Section 1: Crop Biology and Disease Epidemiology

XYLEM CHEMISTRY MEDIATION OF RESISTANCE TO PIERCE'S DISEASE

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Reporting Period: The results reported here are from work conducted from September 2003 to September 2004.

ABSTRACT

INTRODUCTION

Xylella fastidiosa (*Xf*) is a Gram-negative xylem-limited bacterium that causes Pierce's disease (PD), plum leaf scald, phony peach disease, almond leaf scorch, citrus variegated chlorosis, and numerous *other* diseases. In susceptible species xylem vessels may get plugged by *Xf* cells and an exopolysaccharide matrix. Vessel plugging results in xylem dysfunction, water stress, and leaf necroses, which are all characteristic of PD. Cell multiplication, formation of aggregates and biofilm are early components of PD that precede visible symptoms. The stimuli for aggregation and biofilm may involve specific plant/bacterium interactions and may involve the nutrient status of xylem fluid. Xylem fluid typically consists of 95 - 98% water; amino acids, organic acids, sugars and inorganic ions are the major components of total osmolality. Recent progress in generating a simple chemically-defined media for *Xf* allows studies of nutritional requirements *in vitro*. We have found that certain chemically-defined media (3G10R and CHARD2) developed in our laboratory promote the development of cell aggregates and biofilm. Aggregation and biofilm formation of *Xf* in vitro is dependent on xylem fluid chemistry. For example, xylem fluid of *Vitis vinifera* induced a high degree of aggregation of *Xf* cells, whereas *V. rotundifolia* did not. We have also found that calcium ($CaCl_2$) also promoted cell aggregation *in vitro.* These results support the calcium bridging hypothetical model that was proposed to explain how *Xf* adheres to xylem vessels (Leite et al. 2002, 2004b), which assumes that the surface of *Xf* cells are negatively charged due to the presence of sulfur in the outer membrane proteins (OMP). Aggregate formation may be facilitated not only by calcium bridging but also by the formation of disulfide bonds in the OMP. The chemistry of xylem fluid may be a function of temperature, fertilization and diurnal/temporal alterations (Andersen and Brodbeck 1989ab, 1991, Andersen et al. 1995, 2004), and pH (Leite et al. 2004b). It is possible that the manipulation of xylem fluid composition, whether it is based on the primary organic compounds, ions or proteins in xylem fluid, is one possible method to affect PD-resistance. The dependence of aggregation and biofilm formation on the nutrient content of xylem fluid and growth media suggests that xylem chemistry is important in the mediation of resistance/susceptibility of PD.

OBJECTIVES

1. Determine the effects of nutrient media and xylem fluid chemistry on *Xf* colony number, bacterial growth, aggregation and biofilm formation of *Xf.*
2. Examine the influence of *Xf* surface chemistry during early stages of *Xf* aggregation and biofilm formation.

RESULTS AND DISCUSSION

Distinct *Xf* aggregation patterns are consistent with modifications in xylem fluid chemistry. Xylem fluid from PD-resistant cultivars (*V. rotundifolia* Noble and Carlos) induced low or no aggregation of *Xf*, whereas susceptible *V. vinifera* cultivars Chardonnay and Chenin Blanc exhibited a high tendency to aggregate (Figure 1A). The number of large aggregates formed after incubation in xylem fluid was highly significant ($P > 0.0001$) as a function of cultivar. X-ray microanalysis showed clearly the difference between calcium and phosphorus concentrations between the most susceptible and the most resistant cultivar. The phosphorus peak was more evident in the PD-resistant cultivar Noble and barely detectable in the PD-susceptible cultivar Chardonnay (Figure 1B). In xylem fluid from California, the ratio of Ca/P for Noble was close to 1, contrasting with a ratio of 14.5 for Chardonnay. Since calcium and magnesium have been implicated as being involved in adhesion and aggregation of *Xf* (Leite et al. 2002), we compared the concentration of calcium, phosphorus and citric acid. The reason for this approach is that phosphorus and citric acid are known to remove calcium and magnesium from a solution by precipitation in the form of complexes or insoluble salts (Van Der Houwen and Valsami 2001). The ranking of resistance of cultivars from California was reflected by the ratios of compounds that either remove divalent cations (phosphorous and citrate) from solution or divalent cations themselves ([P]*[citrate]/[Ca]*[Mg]), (Figure 1C). However, the same ratio ([P]*[citrate]/[Ca]*[Mg]) did not produce consistent results with xylem fluid from Florida plants (Figure 1C), possibly as a consequence of the presence of citrate and/or phosphate stress (Hoffland et al, 1989; Zhang et al. 1997). Florida xylem fluid much less phosphate than California xylem fluid (data not shown). The concentration of calcium to phosphorus and calcium to citric acid were affected by cultivar and location. The effect of a source of calcium ($CaCl_2$) on *Xf* aggregation was tested

in vitro. In Figure 2A, high number of large aggregates was observed in concentrations of $CaCl_2$ above 50 mg/l (Leite et al., 2004a). An exponential curve was obtained by plotting large aggregates versus calcium chloride concentration (Figures 2 A and B).

The pH of xylem fluid samples collected in Florida were consistently acidic, contrary to variable pH readings obtained in California (data not shown). All *V. vinifera* cultivars in California were pH 7.4 and above. Analysis of pectin content showed the most resistant cultivar (Noble) had more pectin than the most susceptible cultivar (Chardonnay) (Figure 2C). Categorized separation of the pectin samples also showed that uronic acids, which are known to bind calcium, were comparatively higher in Noble. A plant with more uronic acids as part of the xylem cell wall could perhaps control levels of free calcium through the so called "egg boxes" (Braccini and Perez 2001). Calcium bridging seems to be critical at the first stages of micro-colony and colony formation. Calcium availability and the number of *Xf* cells within the xylem vessel may influence the amount and size of aggregates formed. Negative surfaces of *Xf* cells (Leite et al 2002) are important for calcium bridging and the number of negative moieties may be associated with strain pathogenicity. Ultimately, cell aggregation by Ca^{2+} may be the trigger for activation of other pathogenicity pathways. Our preliminary results show that the nutrient content of xylem fluid is a significant component for the development of aggregates and biofilm, although the elucidation of the role of specific compounds requires further research.

The minimum xylem fluid-based medium (3G10R) increased the capacity of *Xf* to form biofilm compared to PW+ medium and reduced the number of cells in the planktonic state (Leite et al. 2004a). This knowledge allowed us to design experiments and investigate the role of each component for *Xf* growth and biofilm formation. The approach adopted was the deletion of one component at a time from the original 3G10R, such as: $MgSO_4$, phenol red, L-glutamine, glucose, ferric pyrophosphate and glutathione. Glutamine is an indispensable medium component for *Xf* (Davis et al. 1981; Chang and Donaldson 1993, Lemos et al 2003, Leite et al. 2004a, Almeida et al. 2004). Glutamine is the most abundant amino acid in *Vitis* xylem fluid (Andersen and Brodbeck 1989, 1991, Andersen et al. 1995Leite et al. 2004a, Ishida et al. 2004). Glucose is found in lower concentration in xylem fluid of grapevines (Andersen and Brodbeck, 1989, 1991). *Xf* can survive a without a glucose source, as demonstrated by Leite et al. (2004a) and this work. *Xf* apparently does not use the pathway to metabolize glucose (Facincani et al. 2003). Iron homeostasis is an important process regulating the expression of genes involved in pathogenicity in bacteria (Vasil and Ochsner 1999, Simpson et al. 2000). In *X. campestris* pv *campestris*, iron-uptake genes are essential for the induction of the hypersensitive response (HR) in non-host plants and disease symptoms in the host plant (Wiggerich and Pühler 2000).

After 4 days of incubation, the ratio of *Xf* biofilm/cells in suspension (planktonic form) was greatly enhanced when glutamine or glucose were withheld from the 3G10-R formulation. (Figure 3A). After 14 days the absence of other elements such as $MgSO_4$, ferric pyrophosphate and glutathione also seemed to promote biofilm formation. Recently, Ishida et al. (2004) observed that *Xf* in the presence of grapevine xylem fluid (*V. vinifera* cv. Chardonnay) formed biofilm after only 30 min of incubation. Leite and collaborators (2004a) showed that in xylem fluid based-minimum media *Xf* formed more biofilm than in PW^+ (a complex and nutritional rich medium). These results suggest that *Xf* was able to form biofilm in response to a microenvironment provided by xylem fluid (a mixture of proteins, amino acids, organic acids, sugars and inorganic ions) and *Xf* could use aggregation as survival mechanism in a nutrient poor growth medium. When *Xf* cells were incubated in deionized water or in oxidized glutathione less biofilm was observed (Figure 3B). In contrast, in a reduced environment such as in the presence of the antioxidants 1,4-dithithreitol (DTT) or reduced glutathione, an increase in biofilm formation was observed (Figure 3B). The details of the mechanism of action of these antioxidants in the *Xf* adhesion process are unknown; however, the calcium bridging hypothetical model supports these findings (Leite et al. 2002). Studies directed toward cell aggregation and biofilm formation may help to understand critical elements of pathogenicity.

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FUNDING AGENCIES
Funding for this project was provided by the CDFA Pierce's Disease and Glassy-winged Sharpshooter Board.

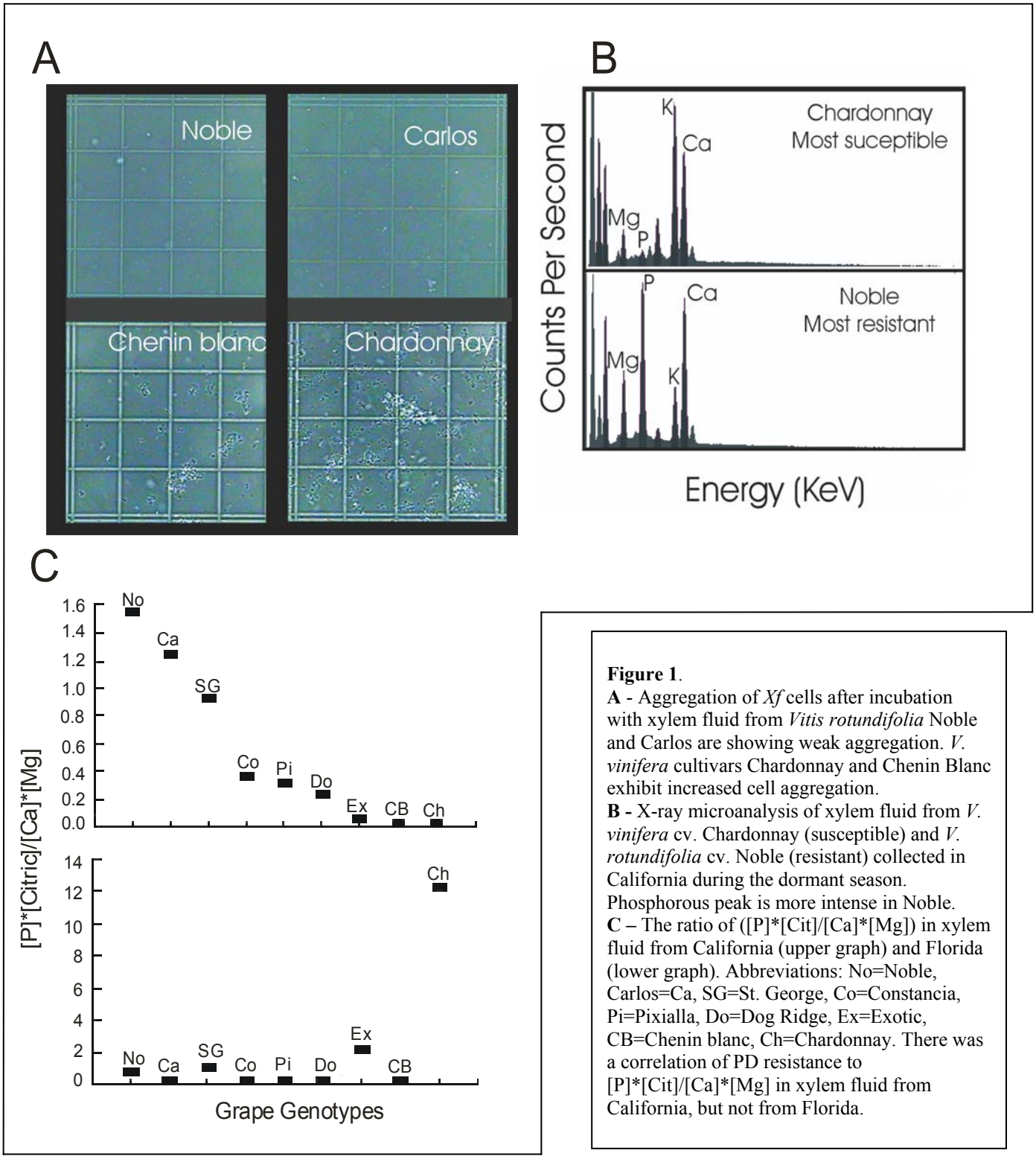

Figure 1.
A - Aggregation of *Xf* cells after incubation with xylem fluid from *Vitis rotundifolia* Noble and Carlos are showing weak aggregation. *V. vinifera* cultivars Chardonnay and Chenin Blanc exhibit increased cell aggregation.
B - X-ray microanalysis of xylem fluid from *V. vinifera* cv. Chardonnay (susceptible) and *V. rotundifolia* cv. Noble (resistant) collected in California during the dormant season. Phosphorous peak is more intense in Noble.
C – The ratio of ([P]*[Cit]/[Ca]*[Mg]) in xylem fluid from California (upper graph) and Florida (lower graph). Abbreviations: No=Noble, Carlos=Ca, SG=St. George, Co=Constancia, Pi=Pixialla, Do=Dog Ridge, Ex=Exotic, CB=Chenin blanc, Ch=Chardonnay. There was a correlation of PD resistance to [P]*[Cit]/[Ca]*[Mg] in xylem fluid from California, but not from Florida.

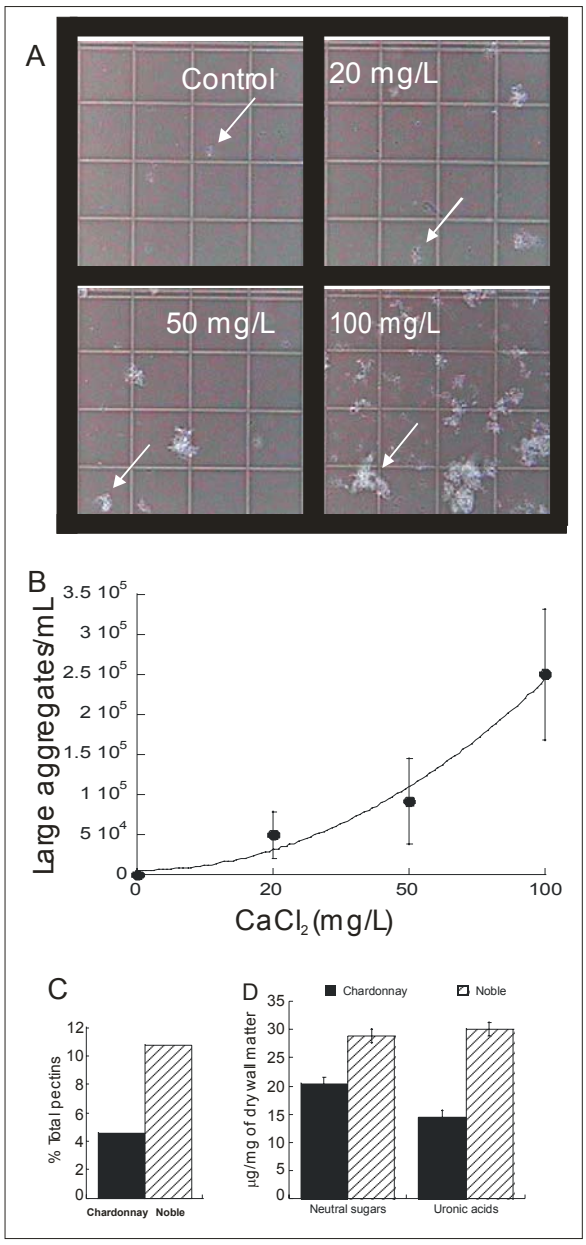

Figure 2.
A - Increasing numbers of *Xf* aggregates in different $CaCl_2$ concentrations. The number of aggregates was visualized in Neubauer chamber: Highest aggregation is at 100 mg/l.
B - Calcium induces aggregation at same concentration range found within the xylem fluid. Points are mean ± S.D., n = 6.
C - Total pectin content of cultivars Noble and Chardonnay.
D - Concentration of neutral sugars and uronic acids in dry cell wall matter of cultivars Noble and Chardonnay.

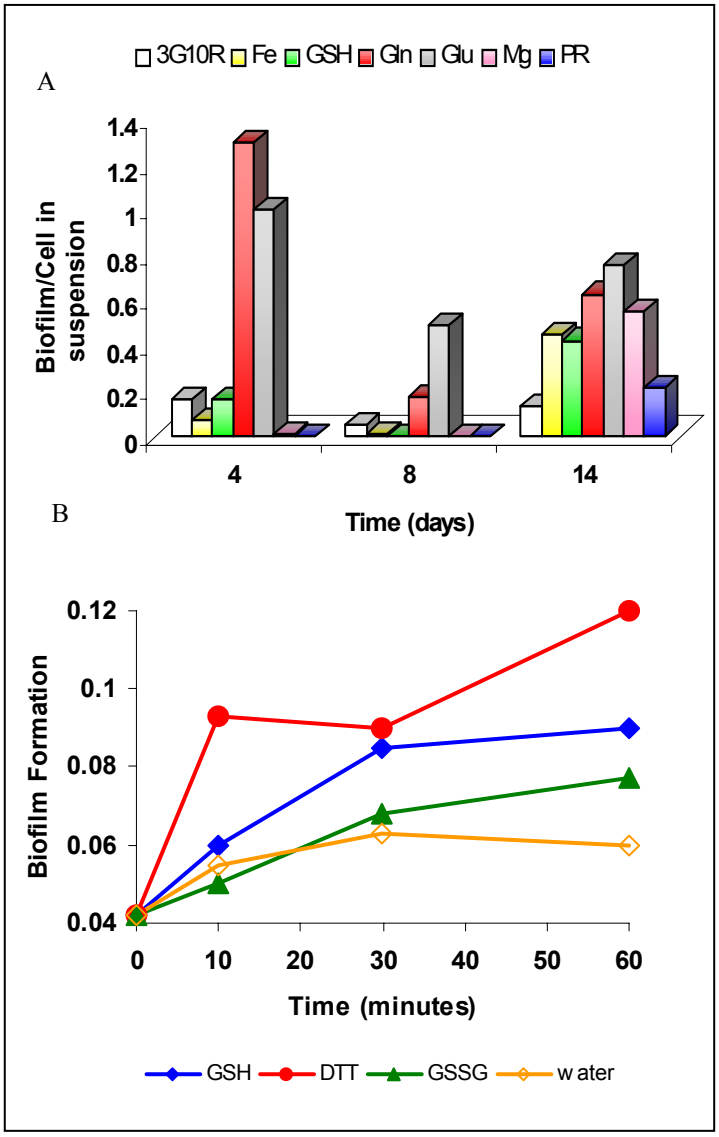

Figure 3.
A - Ratio of Biofilm/Cell in suspension after incubation of *Xf* cells for 4, 8 and 14 days in media generated after deletion of either ferric pyrophosphate (Fe), glutathione (GSH), L- glutamine (Gln), glucose (Glu), $MgSO_4$ (Mg) or phenol red (PR) from 3G10R.
B - Effect of antioxidants on biofilm formation by *Xf* after variable periods of time. The antioxidants used were 1,4-dithithreitol (DTT) 60 mM and glutathione (20 mM) in the reduced (GSH) and oxidized (GSSG) forms. Treatment with distilled water was used as control.

SIGNIFICANCE OF RIPARIAN PLANTS IN THE EPIDEMIOLOGY OF PIERCE'S DISEASE

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Reporting Period: The results reported here are from work conducted from April 1, 2003 to September 30, 2004.

ABSTRACT
The goal of this research is to evaluate the significance of riparian hosts in the epidemiology of Pierce's disease (PD) in the North Coast grape-growing region of California. The first objective is to examine the epidemiological role of seasonal changes in *Xylella fastidiosa* (*Xf*) concentrations in riparian hosts. Among systemic riparian hosts, differences in seasonal *Xf* concentrations and *Graphocephala atropunctata* (blue-green sharpshooter, BGSS) feeding preference affect their importance as *Xf* reservoirs. Temperature affects *Xf* concentrations in plant hosts and, in turn, *Xf* concentrations affect the probability of a BGSS acquiring *Xf* while feeding on an infected plant. We focused on *Xf* concentrations in five systemic hosts: *Rubus discolor* (Himalayan blackberry), *R. ursinus* (California blackberry), *Sambucus mexicana* (blue elderberry), *Vinca major* (periwinkle), and *Vitis californica* (California grapevine). We needle inoculated potted plants of California grape, California blackberry, Himalayan blackberry, blue elderberry, and periwinkle in the greenhouse and transferred all infected plants to two sites in the North Coast (Napa County, Mendocino County). *Xf* was not detected in the majority of plants after several months in the field (from July to Oct. 2003), except for periwinkle which maintained a high number of infected plants through all seasons. *Xf* concentrations were highest in periwinkle in all seasons and at both sites, and were sufficient for BGSS acquisition year-round. California grape and Himalayan blackberry supported *Xf* concentrations that were sufficient for BGSS acquisition in autumn and summer, but not in spring. These results suggest that BGSS likely acquires *Xf* from riparian hosts in autumn, instead of spring.

INTRODUCTION
Past research (Purcell 1976, 1981) demonstrated the direct relationship between incidence of Pierce's Disease (PD) in *Vitis vinifera* and proximity to riparian plants bordering vineyards in the North Coastal grape-growing region of California. Vineyard rows closest to riparian plants (plant species that occupy the banks of rivers and streams) experience the heaviest losses, but there are fewer diseased vines farther away from riparian plants. Riparian habitat adjacent to vineyards contains plants that are feeding and breeding hosts for *Graphocephala atropunctata* (blue-green sharpshooter, BGSS), the most efficient vector of PD in the Napa Valley (Hewitt et al 1949, Purcell 1975). Not only do riparian plants provide habitat for BGSS, but some are also reservoir hosts of the PD strain of *Xylella fastidiosa* (*Xf*) (Freitag 1951). A variety of common riparian plants are capable of maintaining *Xf* infections without expressing disease symptoms. Purcell and Saunders (1999) found that *Xf* populations are, generally, lower in riparian hosts than in grapevines. The ability of *Xf* to multiply and spread within a plant host varies from species to species. After screening several breeding hosts of BGSS for systemic movement of *Xf*, Hill and Purcell (1995) found that only two tested, *Rubus discolor* (Himalayan blackberry) and *V. vinifera*, supported systemic infections. These results imply that some riparian hosts are more important than others as *Xf* reservoirs.

Interactions among BGSS, *Xf*, and their host plants are likely to vary with season. Seasonal changes in BGSS flight activity have been documented (Feil et al 2000). Seasonally variable levels of plant hormones (Hopkins 1985) and changes in temperature (Feil and Purcell 2001) can have major effects on *Xf* concentrations in host plants. *Xf* concentrations change on a seasonal basis in *V. labrusca* (Hopkins and Thompson 1984), and they are lower in *V. vinifera* grown at cooler temperatures (Feil and Purcell 2001). Transmission by BGSS is influenced by *Xf* concentrations in the plant host; the higher the concentration, the higher the probability of BGSS acquiring *Xf* (Hill & Purcell, 1997). Therefor, we might expect that seasonal fluctuations of *Xf* concentrations may influence the spread of PD to grapevines by affecting the proportion of BGSS that acquire *Xf* when feeding on riparian hosts.

OBJECTIVES
The goal of this research is to evaluate the significance of riparian hosts in the epidemiology of PD in the North Coast. Among systemic riparian hosts, differences in seasonal *Xf* concentrations and vector feeding preference affect their importance as *Xf* reservoirs. Temperature affects *Xf* concentrations in plant hosts; *Xf* concentrations, in turn, affect the probability of *Xf* acquisition by BGSS. Probability of *Xf* acquisition is also influenced by how attractive a host is to BGSS; a systemic riparian host that is fed upon more frequently will likely serve as a more significant source of *Xf*. The first objective is to examine the epidemiological role of seasonal *Xf* concentration fluctuations in riparian hosts in the field, where plants are

subject to seasonal temperature changes. We focused on *Xf* in five systemic hosts: *Rubus discolor* (Himalayan blackberry), *Rubus ursinus* (California blackberry), *Sambucus mexicana* (blue elderberry), *Vinca major* (periwinkle), and *Vitis californica* (California grapevine). Future research will focus on BGSS feeding preference.

We tested the hypothesis that seasonal *Xf* concentrations were the same among riparian hosts at two sites in the North Coast (Napa County and Mendocino County). In July 2003, we transferred infected plants from the greenhouse to the field. Plants were in 3-gallon pots and were surrounded by a fine-mesh screen enclosure. *Xf* concentrations were estimated seasonally from petioles and stems, using dilution plating and real-time PCR. The effects of plant species, season, and location on *Xf* concentrations were determined using an analysis of variance (ANOVA). Results from the two quantitation techniques were analyzed separately. Real-time PCR results for summer 2004 are still in progress. This report focuses on culture data.

RESULTS

Xf infections were not sustained in the majority of infected plants, not just from autumn 2003 to the following summer 2004, but also in the short time between transferring infected plants to the field (July 2003) and the first culture attempt in autumn 2003 (Table 1). These results suggest that both cold winter temperatures and high summer temperatures can negatively affect *Xf*. Very few *Xf*-positive plants were detected by culture in winter 2004 and spring 2004 (Table 1). In fact, periwinkle was the only species with enough *Xf*-positive stems in winter 2004 and spring 2004 to make comparisons with summer 2004 data. Based on culture results, *Xf* concentrations were consistently high in periwinkle stems at both sites, with the highest detected in summer (Figure 1), suggesting that periwinkle is an excellent reservoir host, year-round.

Figure 1. Seasonal *X. fastidiosa* concentrations in periwinkle stems, as determined by culture. See Table 1 for sample sizes. Error bars are 95% confidence limits.

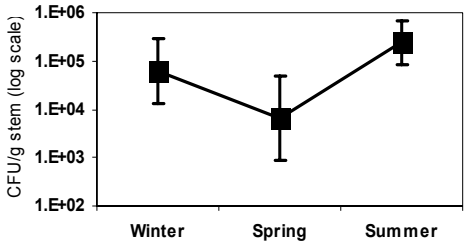
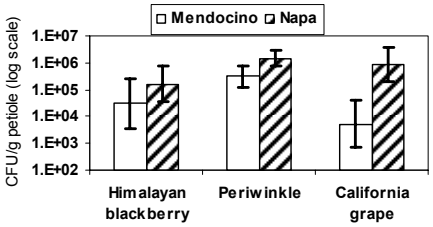


Figure 2. *Xylella fastidiosa* concentrations in petioles of riparian hosts at two North Coast sites, as determined by culture. Means are averages of autumn 2003 and summer 2004 data. See Table 1 for sample sizes. Error bars are 95% confidence limits.



In autumn 2003 and summer 2004, when there were more *Xf*-positive plants detected by culture than in winter 2004 and spring 2004, we found subtle relative differences among plant species at the two sites. For example, in Mendocino, California grape petioles had significantly lower *Xf* concentrations than periwinkle petioles, but there were no significant differences in *Xf* concentrations among periwinkle, Himalayan blackberry, or California grape in Napa (Figure 2). Assuming our results reflect that of naturally-established riparian hosts, spring *Xf* concentrations are sufficient for acquisition by BGSS in periwinkle, but not in any of the other riparian hosts tested.

Our autumn culture attempt coincided with the increased flight activity of young adult BGSS, which peaks in mid summer and remains high through early autumn (Feil et al 2000). Assuming BGSS feeds on California grape, Himalayan blackberry, and periwinkle in early autumn, *Xf* may be transmitted from infected riparian plants to adjacent vineyards before the end of the growing season. While late season infections of grapevines are unlikely to result in chronic disease before infected canes are pruned out in winter (Purcell 1981), young adult BGSSs that acquire *Xf* in autumn and survive the winter are still capable of transmitting *Xf* the following spring.

Real-time PCR results detected more *Xf*-positive plants and much higher *Xf* concentrations than culture results for all species, seasons, and tissues (*data not shown*). Real-time PCR is more sensitive than culture, so we expect that this DNA-based technique would detect *Xf*-positive plants with very low *Xf* concentrations. Detection of higher *Xf* concentrations by real-time PCR as compared to culture is likely a function of *Xf* cells being sticky; the assumption that one colony results from one cell when culturing *Xf* likely leads to underestimates of actual *Xf* concentrations.

Table 1. Number of tested plants confirmed infected with *X. fastidiosa*, as determined by culture and real-time PCR from petioles and stems for four consecutive seasons. All plants were infected upon transfer from the greenhouse to the field in July 2003.

			# *X. fastidiosa*-infected plants			
			Culture		Real-time PCR	
Season	Location/ species	Plants tested	Petiole	Stem	Petiole	Stem
Autumn 2003	Mendocino					
(Oct. 1-22)	Himalayan blackberry	30	3	NC[y]	8	NC
	California blackberry	30	0	NC	0	NC
	Blue elderberry	30	0	NC	4	NC
	Periwinkle	30	28	NC	30	NC
	California grape	30	4	NC	13	NC
	Napa					
	Himalayan blackberry	29	9	NC	13	NC
	California blackberry	30	0	NC	4	NC
	Blue elderberry	28[w]	0	NC	0	NC
	Periwinkle	30	30	NC	30	NC
	California grape	25	5	NC	6	NC
Winter 2004	Mendocino					
(Jan. 27-Feb. 11)	Himalayan blackberry	29	0	4	1	26
	California blackberry	26	0	0	1	12
	Blue elderberry	38	0	0	0	1
	Periwinkle	30	1	6	29	30
	California grape	NC	--	--	--	--
	Napa					
	Himalayan blackberry	29	0	3	2	18
	California blackberry	34[x]	0	0	0	15
	Blue elderberry	34	0	0	0	0
	Periwinkle	30	4	19	29	30
	California grape	NC	--	--	--	--
Spring 2004	Mendocino					
(May 24-June 9)	Himalayan blackberry	30	0	1	0	2
	California blackberry	26	0	0	0	0
	Blue elderberry	37	0	0	0	0
	Periwinkle	26	1	5	14	19
	California grape	29	0	0	0	0
	Napa					
	Himalayan blackberry	29	0	6	1	7
	California blackberry	34	0	0	0	3
	Blue elderberry	34	0	0	0	0
	Periwinkle	19	1	6	8	11
	California grape	24	0	0	0	0
Summer 2004	Mendocino					
(Aug. 2-17)	Himalayan blackberry	34	2	3	IP[z]	IP
	California blackberry	29	0	2	IP	IP
	Blue elderberry	38	0	0	IP	IP
	Periwinkle	30	9	18	IP	IP
	California grape	38	2	5	IP	IP
	Napa					
	Himalayan blackberry	43	3	9	IP	IP
	California blackberry	36	0	2	IP	IP
	Blue elderberry	38	0	0	IP	IP
	Periwinkle	29	21	21	IP	IP
	California grape	40	5	8	IP	IP

[w]For real-time PCR, 24 Blue elderberry were tested.
[x]For culture, 33 California blackberry were tested.
[y]Not collected. In Autumn 2003, all plants were too young to collect stem tissue. In Winter 2004, California grape was dormant.
[z]In progress.

CONCLUSIONS

Riparian Revegetation Management is a method of PD control that focuses on removal of host plants of BGSS and *Xf*, followed by revegetation with native, non-hosts. This method has been shown to reduce local populations of BGSS (*unpublished research*, Dr. Alexander H. Purcell, Division of Insect Biology, UC Berkeley), but its impact on the riparian area as a reservoir of *Xf* has not been quantified. To obtain approval for a Lake and Streambed

Alteration Agreement (1600 permit) from the California Department of Fish and Game, grape-growers develop a management plan that includes characterizing the plant community in the riparian area, targeting individual plants for

removal, and selecting replacement plant species that will provide a similar habitat for wildlife, as a source of shelter, food, and nesting sites. This method has some positive aspects: with lower BGSS populations, fewer insecticide applications are used. Some plants targeted for removal, such as Himalayan blackberry and periwinkle, are invasive weeds. However, removal of riparian vegetation is very disruptive to wildlife, it increases streambank erosion, and some riparian hosts are extremely difficult to eradicate.

Overwintering hosts of *Xf* are thought to play an important role in the epidemiology of PD in providing a source of bacteria for spring infections, especially near vineyards where infective adult BGSS do not survive the winter (Purcell and Saunders 1999). BGSS transmission of *Xf* from riparian plants to grapevines in spring is more likely than mid- or late-season infections to result in chronic disease (Purcell 1981). Given low spring *Xf* concentrations in the riparian hosts we tested, it seems likely that BGSSs acquire *Xf* in autumn instead of in spring.

We found very few *Xf*-positive blue elderberry, California blackberry, and California grape at both sites. Given that these plants were infected upon transfer to the field, it seems that hot temperatures in between transferring them to the field in July and the first sampling date in October were sufficient to prevent *Xf* infections from becoming permanent, especially since the numbers of *Xf*-positive plants among these three species stayed low throughout the rest of the sampling dates at both sites. It is possible that blue elderberry, California blackberry, and California grape do not maintain sufficient *Xf* concentrations for BGSS transmission in the field, even in autumn and summer. Given these findings, it may be more important to focus on Himalayan blackberry and periwinkle for control of PD. The fewer riparian plants removed before revegetation, the less disruption to wildlife habitat.

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FUNDING AGENCIES

Funding for this project was provided by the USDA Agricultural Research Service and the CDFA Pierce’s Disease and Glassy-winged Sharpshooter Board.

GLASSY-WINGED SHARPSHOOTER IMPACT ON ORANGE YIELD, FRUIT SIZE, AND QUALITY

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Reporting Period: The results reported here are from work conducted from July 2002 to October 2004.

ABSTRACT

INTRODUCTION
The California citrus growers needed to know what impact if any the glassy-winged sharpshooter *Homalodisca coagulata* (GWSS) has on fruit yield, size and quality as well as tree vigor. The goals of this project are to determine the usefulness of the management of GWSS to prevent yield loss, fruit size reduction, and degraded fruit quality. First we have to know what impact GWSS has on citrus, and second we need to know how to use currently available materials against the GWSS in IPM programs to prevent potential losses as well as minimizing negative impact to other citrus pests. This information is paramount before we can even begin to incorporate these into conventional IPM programs. Prior to the initiation of this study, we didn't know what the effects of heavy GWSS feeding has on the vigor of citrus trees or fruit yield, size, sugar/acid ratio, peel thickness etc. GWSS suppression in citrus was done to prevent the movement of GWSS into grape under areawide management programs to limit the spread of *Xylella fastidiosa* (*Xf*). Therefore, the focus of this study is to determine the impact of heavy GWSS feeding on citrus yields, fruit size, and quality.

OBJECTIVES
This research was initiated to:
1. Address the impact of GWSS on fruit yield, and distribution of fruit size when GWSS are controlled compared to untreated blocks of Valencia oranges, 'Washington' navel oranges, and grapefruit;
2. Evaluate the effects of high GWSS populations have on fruit quality (sugar/acid ratios, peel thickness, sugar/acid ratio, juice quality, peel texture and firmness, susceptibility to post-harvest disorders) in Valencia and Navel oranges;
3. Evaluate the effects of large GWSS populations have on water stress, nutrient loss (Ca etc.), metabolite loss (amino acids, xylem translocated PGRs) due to xylem feeding and fruit drop and fruit quality, and fruit drop; and 4) Determine if Admire enhances fruit size, tree health and vigor in the absence of GWSS.

RESULTS AND DISCUSSION
The data from the first three seasons of this study indicate that chronic high feeding of GWSS on orange reduces overall yield and size distribution (Hix et al. 2003, Hix et al. 2004). At the beginning of the study, two population levels were established in a 'Washington'navel orange grove. The low population level had essentially 0 GWSS/tree and the high population level trees had more than 1100 GWSS/tree during July, August, and September of 2001, 2002, and 2003. At the beginning of this study there were no differences in the mean number of cartons packed by size distributions (Hix et al. 2003). However, as the influences of GWSS feeding were moved, differences were detected (Figure 1). Navel oranges were harvested from 37 trees within the harvest rows on 8 March, 2004 and sent to the Blue Banner Packing House in Riverside for packout and evaluation 10 March, 2004. Two cartons from two sizes (88 and 113) and two grades (Choice and Export) from each replication (total of 96 cartons) were selected. Trans-Pacific shipment was simulated by storing the 96 cartons at the packinghouse for 21 days at 37 ºF after which time the fruit was sent to KAC for storage at 68 °F for 4 days followed by 55 °F for 5 days. For postharvest evaluation, initial measurements of general appearance, pitting, puff and crease, peel firmness, thickness, color, TA, TSS, and percent juice were taken from a 20 fruit sub-sample at harvest. Fruit were subsequently rated for general appearance, rind pitting, and decay following simulated shipment.

The effects of the high feeding populations of GWSS on navel orange peel nutrient status and metabolism have been consistent for the two years of the study. High GWSS feeding populations significantly reduced peel Ca and Mg concentrations both years of the study: year 1 ($P \leq 0.05$) and year 2 compared to the low GWSS population (control trees treated with Admire) ($P \leq 0.001$) (Table 1). High GWSS feeding populations significantly disrupted N metabolism causing high peel nitrate-N or total N in years 1 and 2, respectively ($P \leq 0.05$). (Note that nitrate-N concentration is lower than that of total N and easier to perturb.) High GWSS feeding populations significantly increased peel arginine and putrescine

concentrations in both years of the study with the magnitude of the difference between the two treatments greater in year 2 ($P \leq 0.05$). High GWSS feeding populations resulted in a numerically higher concentration of proline in year 1 and a significantly higher proline concentration in year 2 ($P \leq 0.05$). In year 1, the yield of the 24 data trees in the high GWSS feeding population treatment has numerically lower than the yield of the 24 control trees treated with Admire (low GWSS feeding population). In year 2, the yield reduction caused by the high GWSS feeding population was approximately 50% and significant ($P \leq 0.05$). The effect of GWSS feeding appears to be cumulative over the two years of the study as the magnitude of the changes tended to increase in magnitude and significance from year 1 to year 2. Although GWSS feeding causes changes in peel Ca, Mg and N status, high levels of feeding and the induced changes occur after maximum peel thickness and, thus far, have not affected external fruit quality. The changes in metabolism induced by GWSS feeding are indicative of tree stress. The increased magnitude and statistical significance of these metabolic changes over the two years of high GWSS feeding pressure is consistent with cumulative stress to the trees.

The rind pitting is seemingly a postharvest disorder and is not caused by direct damage of the GWSS. Pitting was clearly a problem in the May 2003 harvested Newhall 'Valencias', but there were no significant differences in the treated (i.e. low population trees) and the untreated (high population trees) ($F = 0.361$, $P = 0.550$). The low population trees had 34.4% pitting (± 1.23 SEM), whereas the high population trees had 36.5% pitting (± 1.2 SEM) following simulated trans-Pacific shipping as described above. Navel pitting on the Jan. 2003 harvest following simulated trans-Pacific shipment was 3.9% (±0.3 SEM) for the untreated trees (high populations) and 4.1% (±0.5 SEM) on the treated (low or 0 population trees). Prior to shipment simulation, pitting on the navels was 0.03% and 0.01%, respectively. The preliminary information suggests a postharvest physiological problem that's not the result of GWSS xylem feeding behavior. However, this xylem feeding behavior may be contributing significantly to tree (and fruit) stress as discussed below. Navel orange fruit size distribution for the harvest of 2004 was significantly reduced for the high GWSS population trees (Figure 1). Significantly more cartons of fruit sizes 72, 88, 113 and 138 were packed from the low GWSS population trees. When taking into account oranges rejected to the juice line, the overall yield by weight was also higher for the low population trees.

Consistent results were obtained for 'Valencia' and navel orange orchards in two different citrus growing areas, Newhall and Mentone, respectively, in year 1 of the study and for two consecutive years for the navel orange orchard in Mentone. The results confirm that the glassy-winged sharpshooter disrupts the normal basal carbon and nitrogen metabolism of the peel and creates mineral nutrient deficiencies in the peel compared to fruit from trees not under the feeding pressure caused by high populations of glassy-winged sharpshooter. Peel nutrient deficiencies included significantly lower concentrations of Ca, Mg, NO_3-N (analyzed in year 1 only) (Table 1). There was also a significant reduction in Zn in year 2. In all cases, peels of fruit from trees with high populations of GWSS exhibited classic symptoms of stress: high concentrations of arginine and putrescine and, to a lesser degree, praline (Table 2). Accumulation of arginine and putrescine to a greater degree than proline indicates a loss of available carbohydrate. The consistent and persistent symptoms of stress observed for trees under heavy feeding by high population densities of GWSS correlates with losses in yield and fruit size.

One half of the trees in a 'Valencia' orchard in Woodlake, Calif. were treated 27 June 2002, 13 June 2003, and 10 June 2004 with 32 oz of Admire per acre administered through the irrigation system. The treated and untreated areas were five rows wide by 79 trees long and replicated three times each. Twenty trees in each plot were analyzed for total number of fruit and fruit weight. Twenty fruit of size 56 in each plot were used to determine average length, width, and rind thickness, percent juice, sugar/acid ratio, and percent soluble solids.

Table 3 shows that there were no significant differences in fruit number or weight before treatments were applied. In May 2003, in the season after the first Admire treatment, there was still no difference in fruit number per tree, fruit weight or fruit size distribution. In May 2004, after the second Admire treatment, the number of fruit per tree was significantly reduced in the Admire treated trees in 2003 and the fruit size was significantly larger. There was a significantly higher sugar/acid ratio in the fruit from the Admire-treated fruit only during 2003. Thus, we saw no consistent effect of Admire on fruit quality. See Hix et al. 2003, Hix et al. 2004 for 2002 and 2003 results.
See Hix et al. 2003 and Hix et al. 2004 for additional 2003 results.

Table 1. Effect of GWSS population density on peel nutrient status of navel orange.

	Nutrient				
	Year 1 (20 Aug.)			Year 2 (15 Sept.)	
Population density	Ca	Mg	NO_3-N	Ca	Mg
	---------- % ----------		*--- ppm ---*	*---------- ppm ----------*	
High	0.84 b	0.12 b	1292.6	7409 b	1068 b
Low	1.09 a	0.15 a	1536.6	10280 a	1497 a
Significance	*	*	*	***	***

Table 2. Effect of GWSS population density on the metabolism of 'Valencia' and navel orange trees in Newhall and Mentone, respectively, on the accumulation of stress metabolites in peel tissue.

Population density	'Valencia'	Navel	
	Year 1 (15 Aug.)	Year 1 (20 Aug.)	Year 2 (15 Sept.)
	nmol arginine/g fresh wt peel		
High	1319[z]	2429	1092
Low	1210	2271	983
Significance	**	*	*
	nmol putrescine/g fresh wt peel		
High	444	716	517
Low	407	397	272
Significance	*	*	*
	nmol spermidine/g fresh wt peel		
High	43	74	18
Low	40	122	13
Significance	*	*	NS
	nmol proline/g fresh wt peel		
High	8826	8100	8312
Low	9281	8200	7400
Significance	NS	NS	*

[z] Means within a vertical column within a section of the table were separated by Duncan's multiple range test at $P = 0.05$.

[NS, *, **] Not significant or significant at $P \leq 0.05$ or 0.01, respectively.

Table 3. Fruit number, weight and size of 'Cutter' Valencia oranges harvested from Paramount-Rayo (block 49) and processed at Lindcove Research and Extension Center (treated with Admire on 27 June 2002, 13 June 2003 and 10 June 2004).

Treatment	Avg # fruit	Avg fruit weight (lb)	Size 88	72	56
March 2002					
Untreated	473.3a	0.51a	24.7a	30.9a	23.9b
Admire 2F	470.5a	0.48a	21.0b	31.2a	33.7a
May 2003					
Untreated	623.1a	0.50a	18.6a	28.3a	38.0a
Admire 2F	659.0a	0.47a	20.2a	29.0a	32.2a
May 2004					
Untreated	480.1a	0.43b	22.0a	24.2a	19.5a
Admire 2F	404.1b	0.46a	21.0a	24.9a	26.4a

Means within a column followed by the same letter are not significantly different (LSD, $p = 0.05$).

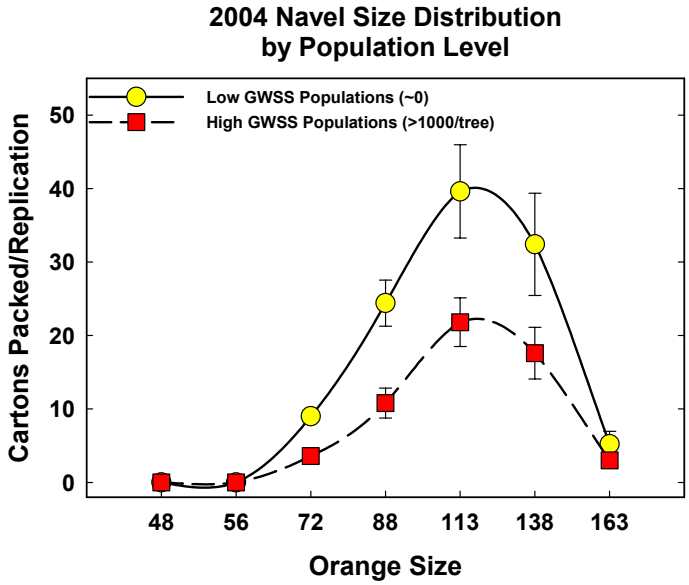

Figure 1. Mean number (±SEM) of cartons packed fresh for market on 10 March 2004. N=5 reps. 1 rep = 37 trees. 902 total cartons packed fresh with 751 cartons were packed from the "low" GWSS trees and 151 were packed from the "high" GWSS trees.

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FUNDING AGENCIES

Funding for this project was provided by the California Citrus Research Board and the University of California Pierce's Disease Grant Program.

LINKING THE MODEL OF THE DEVELOPMENT OF PIERCE'S DISEASE IN GRAPEVINES TO AN UNDERSTANDING OF THE DYNAMICS OF GLASSY-WINGED SHARPSHOOTER TRANSMISSION OF *XYLELLA FASTIDIOSA* TO GRAPEVINES AND GRAPEVINE GENE EXPRESSION MARKERS OF PIERCE'S DISEASE

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Reporting Period: The results reported here are from work conducted from April 1, 2004 to October 1, 2004. (**Note**: This includes work on an earlier "Pierce's Disease Model" research proposal, and work begun on this new proposal.)

INTRODUCTION

For three years, our group has been testing the "steps" in PD development that were proposed in a model.

***Xf* introduction to vessels—>vessel cavitation—> initial water deficit—> *Xf* population increase—> production of enzymes by *Xf*—> cell wall digestion —> oligosaccharide signals —> ethylene synthesis rise—> a "wave" of vessel occlusion beyond the infection site —> collapse of vine water transport—> leaf abscission—>vine death**

In the course of that research, we have shown that xylem vessel obstruction (tyloses, plant cell wall component-derived gels, and, perhaps, bacterial extracellular polysaccharides) and consequent reductions in stem water transport capacity are early consequences of infection with *Xylella fastidiosa* (*Xf*), before bacterial populations are substantial and have spread far from the inoculation point. We have shown that ethylene treatment of vines also triggers vessel obstruction development and reduced water movement and that ethylene emanation from vines may increase following infection. We have also developed data for xylem vessel length distributions in grapevines and shown that *Xf* must pass through vessel pit membranes if the bacterial population is to develop systemically, thus suggesting that digestion of cell wall polymers in the pit membranes is likely to be important to disease spread. These findings are reported in several reports at the annual PD Symposium and, more recently, at disciplinary scientific society meetings and in refereed reports (Stevenson et al., 2004).

Work to retest aspects of our model, those parts relating specifically to the involvement of cell wall breakdown caused by the action of *Xf* enzymes, remain and will be tested in this new proposal (see Objectives). Also to be tested are ideas based on the reports of the studies of others involved in unraveling problems associated with the transmission and spread of PD, within and between grapevines. We will link the anatomical, biochemical and physiological findings from our "model testing" to the work of Cook et al. (), describing genes that are expressed in vines relatively soon after *Xf* infection. We have nothing to report on this aspect of the new proposal. We will also address a question that entomologists and plant biologists generally have differing opinions about. Do vessels cavitate (i.e., become air-filled and, hence, non-functional when the glassy-winged sharpshooter (GWSS) starts or finishes its feeding on a vine? The answer to this question may have important implications regarding *Xf* transmission, GWSS' feeding strategy and spread of the bacteria in an infected vine. Below and in the report from Shackel and Labavitch in these proceedings, we report on the start we have made in addressing this question.

OBJECTIVES

1. To complete testing of our model of PD development in grapevines.
2. To determine whether GWSS feeding on grapevines is accompanied by xylem vessel cavitation.
3. To determine whether the grapevine "regulators" that we have identified as important to development of PD affect the expression of grapevine genes that have been shown to be important markers of *X. fastidiosa* presence/PD infection.

RESULTS

The Path of Xf Movement in the Grapevine Xylem.

In previous reports, we have described tests that indicate the porosity (i.e., the space between the polysaccharides) of vessel pit membranes is between less than 29 nm, much too small to permit passage of *Xf*. We have refined those tests by using colloidal gold particles having diameters of 20 and 5 nm. While the particles are very difficult to see under the microscope, their presence can be readily detected chemically by reacting samples containing the particles with Sigma Chemical Company's "silver enhancer". A segment of grapevine stem is fitted into a tube attached to a valving device that permits introduction of a small volume containing colloidal gold particles to the stem while maintaining pressure on a water line that drives water through the segment. Introduction of food coloring, whose movement through the stem is not impeded by pit membranes, to the system and collection of the water + dye exiting the stem at the distal end indicates that the volume of water needed to move from one end of a 50 cm stem segment is less than 200 µl. Colloidal gold particles with a 5 nm can move through healthy stem segments, particles of 20 nm diameter cannot (Figures 1 & 2). However, when we used a vine that was showing the initial visible symptoms of PD **at its base** (i.e., its older internodes) and tested the movement of colloidal gold particles through a stem segment cut from the younger portion of the stem that had not yet begun to show PD symptoms, particles of 20 nm diameter moved through the xylem and were collected at the distal end. These results suggest that a decreased pit membrane polymer integrity, hence increased porosity, occurs in healthy appearing stems on infected vines. These results must be confirmed and expanded on (for instance, how much larger are the pores in infected vines?), but they suggest that pit membranes are being opened up in infected vines, perhaps to permit the systemic movement of *Xf*).

The Importance of Xf's Cell Wall-degrading Enzymes to PD Infection.

UC Davis Plant Pathology Ph.D. candidate Caroline Roper and Carl Greve have been working to characterize the gene products of the putative polygalacturonase- (PG) and β-1,4-glucanase- (BGase) encoding sequences identified in the *Xf* genome. In a report at last year's PD Symposium (Labavitch and Matthews, 2003) we reported on Caroline's work with cloning of bacterial "PG" and "BGase" sequences and expression of the cloned genes in *E. coli*. Apparently the *E. coli*-produced proteins are accumulating in inclusion bodies. This is not an uncommon result with this sort of approach, but it does increase the problems with isolating and characterizing the enzymes produced. The work with BGase has proceeded more rapidly. We have shown that the *E. coli* lines expressing the cloned sequences have been induced to express the genes and proteins showing BGase and PG activity have been isolated from them. We are using a combination of protocols to enhance expression and isolation (extraction, solubilization and proper refolding of the expressed proteins) of the two enzymes for use in testing the ability of these enzymes to facilitate *Xf*, polystyrene bead and colloidal gold particle movement through healthy vines. In the meantime, we have initiated an interaction with Novozymes (a Danish biotech enzyme company with a research operation in Davis) to obtain pure microbial PG and BGase for preliminary tests of the impact of these enzymes on pit membrane porosity. The role of PG is particularly important with regard to understanding the reported control of PD development in grapevines that is provided by transgenic expression of a PG-inhibiting protein (PGIP) in *V. vinifera* (The work of Dr. Cecilia Aguero, reported in Meredith and Dandekar, 2002 and 2003; also Aguero et al., 2004 *in press*).

While we are still working to isolate and characterize the *Xf* PG and glucanase, we have developed a strong case for the importance of PG in PD development. Roper has generated an *Xf* mutant with its PG gene knocked out by homologous recombination insertion of a defective PG sequence. Pathogenicity tests with the wild type and PG-deficient *Xf* strains have shown that while the PG-deficient bacteria are able to persist in grapevines they are much less virulent (Figure 3, Table 1) (Roper et al., 2004). We continue to test the relative pathogenicity of these strains and hope to identify specific differences in the gene expression responses of grapevines to inoculation with them.

Is Vessel Cavitation Associated with GWSS Feeding on Grapevines?

In a separate report in the proceedings for this symposium, Shackel and Labavitch report on the work of Plant Biology Ph.D. candidate Alonso Perez indicating that the cavitation of vessels can be readily seen in MRIs of grapevine stems (also in Perez et al., 2004). Elaine Backus and her colleagues at the USDA research facility in Parlier are now set up to perform EPG monitoring of sharpshooter feeding in their new lab. Our groups have been interacting to combine MRI and EPG monitoring with testing for acoustical emissions from grapevines (an indicator of vessel cavitation events) to ask whether vessels cavitate during insect feeding. These tests will probably be made in the first half of 2005.

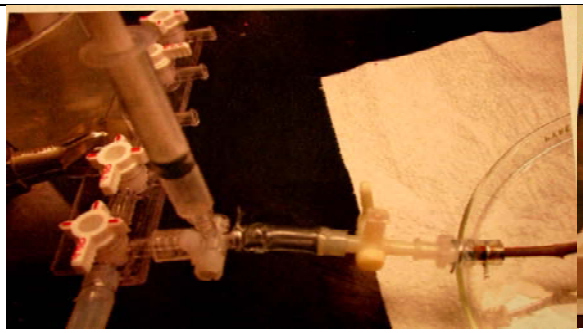

Figure 1. Proximal end of a cut grapevine stem fitted into the valving system that allows (1) pressure driven passage of water through the stem and (2) introduction of colloidal gold particles.

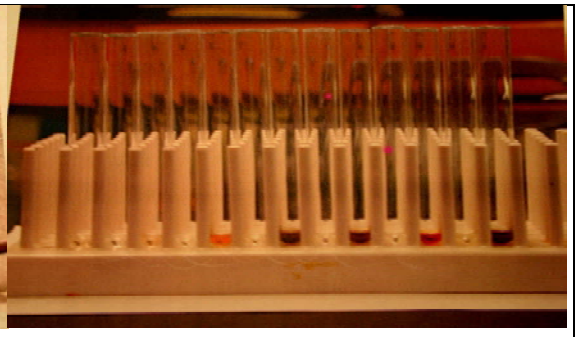

Figure 2. A series of tubes, each containing a 100 ul volume of the water forced through the stem shown in Fig. 1 and collected (left to right, in sequence) as it flowed from the distal end. The presence of gold particles is revealed by the use of silver enhancer.

Table 1. Disease severity of greenhouse-grown grapevines inoculated with wild-type *Xf* (Fetzer isolate), the Fetzer isolate with mutated (non-functional PG sequence) and water. Plants were rated for visual symptoms from 0 to 5, with 0=healthy (no symptoms) and 5=dead. 10 plants evaluated per treatment.

Time post-inoculation	Vines inoculated with:		
	WT *Xf*	PG-*Xf*	Water
12 weeks	0.56	0	0
13 weeks	1.22	0	0

Figure 3. 'Chardonnay' grapevines inoculated 13 weeks previously with, left to right, the Fetzer *Xf* isolate, the Fetzer isolate with its PG gene knocked out, and water. Note the differences in disease symptoms. See Table 1.

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FUNDING AGENCIES

Funding for this project was provided by the USDA Animal and Plant Health Inspection Service and the CDFA Pierce's Disease and Glassy-winged Sharpshooter Board.

THE CONTRIBUTION OF THE PECTIN-DEGRADING ENZYME POLYGALACTURONASE (PG) IN TRANSMISSION OF *XYLELLA FASTIDIOSA* TO GRAPE AND USE OF PG-INHIBITOR PROTEINS FOR TRANSGENIC RESISTANCE TO PIERCE'S DISEASE

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Reporting period: The results reported here are from work conducted from April 1, 2004 to October 10, 2004. (**Note**: This includes work done prior to CDFA approval of this new project.)

INTRODUCTION

Pierce's disease (PD) develops because (1) inoculative glassy-winged sharpshooters (GWSS) feeding on grapevines transfer *Xylella fastidiosa* (*Xf*) bacteria into the vine, and (2) the *Xf* population in the vine's water-conducting cells increases and spreads throughout the vine, triggering a set of responses that result in vine collapse and death. Our work on PD development thus far has focused on the spread of the bacteria once they have been introduced into the vine. The cell wall polysaccharide "fabric" of the pit membranes that separate xylem vessels from one another has interpolymer gaps (referred to as cell wall "porosity") that are substantially smaller than *Xf* cells. Thus, the systemic spread of *Xf* is likely to be facilitated by the action of enzymes that digest some of the pit membrane's constituent polysaccharides. Plant cell wall digestion is a common aspect of the biochemistry of most plant interactions with fungal and bacterial pathogens (Powell et al., 2000). In the report describing our continuing work to test a hypothetical model of PD development (Labavitch et al., 2001 & 2002,; Labavitch and Matthews, 2003 and Labavitch et al. in these Proceedings), we have described studies to determine whether *Xf* genes presumed to encode cell wall-degrading enzymes actually do encode the polygalacturonase (PG) and β-1,4-glucanase that their sequences predict. Apparently they do. The work of Dr. Cecilia Aguero (Meredith and Dandekar, 2002 & 2003; Aguero et al., 2004) shows that transgenic grapevines that express the pear fruit gene that encodes a PG-Inhibiting Protein (PGIP) show slower and reduced symptom development, following needle inoculation, than do untransformed grapes. We presume that this is a consequence of the PGIP's inhibition of an *Xf* PG that is crucial for bacterial spread through the vine

As a follow up to work we are doing on plant-insect interactions, we have identified glucanase and PG activity in protein extracts of homogenized GWSS heads. We presume that the enzymes were located in the insect's salivary apparatus and represent some of the proteins in GWSS salivary secretions. If GWSS penetrates grapevine tissues and inserts its stylets in the water-conducting cells of the vine using only mechanical force, why should the saliva of the insect contain PG and other cell wall-degrading enzymes? Dr. Elaine Backus, co-PI on this proposal suggests that the salivary enzymes are important contributors to the insect's feeding success, both in penetration and in correct stylet placement. If this is correct, and if the pear PGIP that has been introduced into transgenic grapevines inhibits the GWSS PG, then the transgenics should also be less susceptible to *Xf* transfer from the insect than untransformed vines.

The Objectives of our work in this proposal are to obtain PG enzyme from both GWSS and *Xf*, and determine the extent to which PGIP inhibits the PGs from the bacteria and insect. Several PGIPs with differing abilities to inhibit PGs from various fungal plant pathogen sources are known (Stotz et al., 2000). If we find that pear PG inhibits either the *Xf* or GWSS PG, or both, continuing research will screen PGIPs from other sources with the intent of identifying an inhibitor with maximal ability to slow infection and disease development in grapevines.

OBJECTIVES

1. To determine whether the pectin-degrading enzyme of *X. fastidiosa* contributes to the systemic spread of the bacterial population in inoculated grapevines (1st priority)
2. To determine whether the pectin-degrading enzyme(s) in the salivary secretions of GWSS contributes to inoculation success of *X. fastidiosa* into grapevines (2nd priority)

RESULTS

This is a new project and funding was only recently received to begin work on specific Objectives. However, because the project is an extension of other PD research (that of others as well as our own) we have some relevant results to present in this progress report.

Grapevines for Testing.

Dr. Cecilia Aguero has teamed with Profs. Meredith and Dandekar to generate transgenic *V. vinifera* (cultivars 'Thomson Seedless' and 'Chardonnay') expressing the pear fruit PGIP gene. These vines accumulate PGIP protein in tissues and in the xylem sap and show decreased susceptibility to infection by *X. fastidiosa* (Aguero et al., 2004). These vines will be the key biological material for testing in the work of this proposal. Dr. Aguero has expanded the populations of these vines to provide the plant material that we will need.

GWSS Cell Wall-digesting Enzymes.

David Morgan has provided to the Labavitch lab several samples of killed GWSS for biochemical analysis. The best samples to examine for their enzyme complement will be excised insect salivary glands and a large-scale collection/dissection "party" is planned for later in the year. In the meantime, we have isolated insect heads, homogenized them in a protein extraction buffer (1M NaCl in 0.1M NaAcetate, pH 5.5), stirred the homogenate at room temperature for 3 h in the presence of protease inhibitors (2% v/v Sigma inhibitor mix) and collected the soluble protein-enriched supernatant following centrifugation at 15,000 x g for 15 min. The extracts are then assayed for PG and β-1,4-glucanase activities using standard radial diffusion assays.

The PG content of the GWSS head protein extracts we have prepared thus far has been quite variable, often rather low. We will wait until we have obtained a substantial number of isolated GWSS salivary glands to attempt the PG purification. However, because the β-1,4-glucanase (BGase) activity has been substantial in all extracts, we have tested many of our insect enzyme purification approaches with the glucanase and made excellent progress.

Protein isolated (as above) from excised heads of 40 GWSS was chromatographed on Concanavalin A Sepaharose. While the protein did not bind "absolutely" to the lectin column, its passage was retarded somewhat. Over 65% of the protein in the extract eluted rapidly from the column, while 90% of the BGase activity was delayed, thus giving a useful first purification step. The active fractions from this step were pooled and subjected to size-exclusion chromatography (SEC) on a Sephacryl S-200 column, This step removed an additional 20% of the protein while allowing us to recover a peak of BGase representing 35% of the initial activity. The final purification involved passage of the pooled, SEC-purified BGase through a Q-Sepharose anion exchange column, eluting first with 5 column volumes of 0.05M Tris-HCl (pH 7.0) and then a linear gradient (0 to 1M NaCl in the Tris-HCl). The elution of the BGase activity was retarded on this column, emerging as a clean peak of activity corresponding to a protein peak. The fractions with BGase activity were pooled , concentrated and run on an SDS-PAGE gel to determine its protein species distribution. A single protein was seen when 40mg of protein was subjected to electrophoreseis, suggesting that a BGase protein had been substantially (or, perhaps, absolutely) purified. The protocols that we have developed for the GWSS BGase should prove useful when we have substantial GWSS to work with.

Work for the Coming Year.

Our plan is to obtain PG-active proteins from GWSS and *Xf*, purify them and test for inhibition by PGIP. In addition, we will monitor the relative infection of control and pear PGIP-expressing transgenic grapevines by GWSS carrying *Xf,* to assess PGIP's contribution to resistance to bacterial transmission from the insect.

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FUNDING AGENCIES

Funding for this project was provided by the CDFA Pierce's Disease and Glassy-winged Sharpshooter Board.

CHARACTERIZATION AND IDENTIFICATION OF PIERCE'S DISEASE RESISTANCE MECHANISMS: ANALYSIS OF XYLEM ANATOMICAL STRUCTURES AND OF NATURAL PRODUCTS IN XYLEM SAP AMONG *VITIS*

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Reporting Period: The results reported here are from November 2003 to October 2004.

ABSTRACT
This research tests the hypothesis that Pierce's disease (PD) resistance is due to the presence of chemical factors, e.g. anti-microbial compounds expressed in the xylem sap that suppress *Xylella fastidiosa (Xf*) and /or are due to anatomical features of the xylem, e.g. pit membrane that restrict *Xf*'s mobility in xylem. A wide range of PD resistance from various genetic backgrounds of *Vitis* species was selected for this study. To determine if pathogen movement *in xylem* is related to anatomic structure, an inter-grafting method was used to evaluate the movement of *Xf* across between PD susceptible and resistant stems. SEM and quantitative PCR were used for this study. To test the effect of xylem sap, an *in vitro* bioassay method was developed. The preliminary bioassay results suggest that xylem saps from PD resistant grapes may have effect when the test was compared with the sap from *V. vinifera cv.* Chardonnay.

INTRODUCTION
Plants have evolved a variety of resistance and tolerance mechanisms against biotic stress. This rich diversity results in part from an evolutionary process driven by selection for acquisition of defense compounds against microbial attack or insect/animal predation. As pesticide use becomes more restricted, it becomes increasingly important to explore and utilize compounds from plant's natural defense systems. Like many other plants, grape species are very diverse. Many *Vitis* species, *V. aestivalis*, *V. arizonica, V. shuttleworthii, V. simpsonii, V. smalliana*, are highly resistant to PD, as have the muscadine species*, Muscadinia munsoniana and M. rotundifolia*. Understanding and utilizing natural defense mechanisms is a critical component of crop improvement. The ultimate solution to PD problems likely relies on host resistance. This research focuses on understanding PD resistance mechanisms in grape species. Although PD resistant species have been identified (Mortensen, et al, 1977), the mechanisms involving resistance have not been well characterized and identified. It appears that PD resistance mechanisms vary – some resistance mechanisms could be related to anatomical aspects while others may be related to xylem chemistry. This research will examine the physiological and anatomical basis of PD resistance. We selected the following grape species to study PD resistance: *V. arizonica*, *V. aestivalis, V. candicans, V. champinii, V. labrusca, M. munsoniana, V. riparia, M. rotundifolia, V. rufotomentosa, V. shuttleworthii, V. simpsonii, V. smalliana, V. tiliifolia,* and *V. vulpina*. Given the fact that these species were derived from various genetic backgrounds and different origins, it is expected that the mechanisms of PD resistance may be different among grape species. *Xylella fastidiosa* is xylem limited and kills vines by inducing or creating vessel blockage leading to disease (Goodwin et al 1988a, 1988b). The pathogenesis of *Xf* appears to be dependent upon its ability to multiply in the xylem vessels and move systematically across vessels. Therefore, the mechanisms of host resistance may act to physically eliminate *Xf* movement or chemically suppress population development, or both. This proposal attempts to determine whether PD resistance is because: 1) anatomical features of the xylem (e.g. pit membrane) eliminate *Xf*'s mobility; 2) chemical compounds (e.g. anti-microbial activity) present in xylem sap suppress *Xf.*

OBJECTIVES
1. Develop an *in vitro* bioassay to determine the roles of compounds present in PD resistant species. Chemically characterize the composition of xylem and identify compound(s) that may contribute to antimicrobial effects which prevent or suppress *Xf* colonization.
2. Examine xylem structure related PD resistance. Use an inter-graft technique to examine the correlation between pathogen movement and xylem anatomy features.

RESULTS
1. Table 1 presents a list of grape species used for bioassays of xylem sap. A 4 inch diameter x 20 inch pressure chamber (PMS Instrument Co., Corvallis, OR) was used to collect xylem sap from shoots. The chamber pressure was gradually increased to 1,000 – 2000 kPa. On average 0.5 to 2.0 ml xylem sap was collected from each sample. Sap collected from infected and non-infected plants was used for bioassays. The xylem sap was first filtered through a 0.22 micron nylon filter. Two bioassays were conducted. The first bioassay was on PW agar medium on which a piece of filter paper saturated with sap solution was placed onto growing *Xf*. Filter paper saturated with 200 µm Tetracycline or water was used as positive and negative controls, respectively. Another bioassay was carried out by directly culturing *Xf* in xylem sap for 10 days prior to spreading sap on a PW plate to check colony formation. Xylem sap from Chardonnay, a PD-susceptible cultivar was used as a positive reference. Using both methods, we screened xylem saps collected from early spring and summer. No inhibitory

effects were observed from the xylem saps collected from early spring. Currently, we are working on the saps collected from growing season. Our preliminary bioassay results indicate that sap from *M. rotundifolia* appears to have effect on *Xf* growth compared with the sap from Chardonnay. Additional xylem sap has been collected from *M. rotundifolia* to confirm the result.

2. To evaluate xylem structure related to PD resistance, we designed an inter-graft method to compare *Xf* movement between PD resistant and PD susceptible stems. Table 2 presents the results of graft combinations with susceptible stems connected with a resistant interstock. We used dormant cuttings for most of grafts. However, *M. rotundifolia* and several other PD resistant species are only successfully grafted with herbaceous cuttings. Because of difficulty in completing these grafts only a limited number of graft combinations could be made, others are still processing. The successfully grafted plants were used for the movement experiment. In August, these plants were mechanically inoculated with 20 µl of mixture of Stag's Leap and Beringer strains (OD_{600}=0.249) at the bottom part of the susceptible stem. Two months after inoculation, PD symptoms began to appear in both the top and the bottom of halves of "Chardonnay -9621-15 - Chardonnay" but not in resistant stems in the middle of inter-grafted plants (Figure 1). We are harvesting leaves and petioles from the bottom, middle and top parts of the each plant to determine *Xf* levels. Currently, we are working on xylem structure among these PD resistant species using SEM.

CONCLUSION

We have commenced a study of the anatomical and chemical aspects of xylem that distinguishes PD resistant species. Understanding and utilizing natural defense mechanisms is a critical component of crop improvement, and our studies will help breeders fine tune selection indices and determine whether xylem chemistry or anatomy characters are more closely involved in PD resistance.

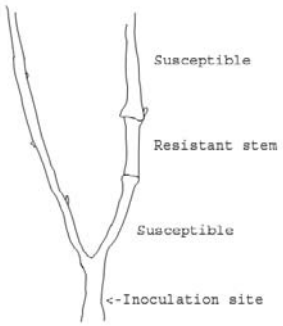
Table 1. List of plants from which the xylem saps were extracted for *in vitro* bioassay.

Resistant species and hybrids

V. arizonica
V. candicans
V. champinii
V. rufotomentosa
V. shuttleworthii Haines City
V. simpsonii
S. smalliana
V. tiliifolia
*M. rotundifoli*a Cowart
V. rupestris Metallique
V. girdiana
V. monticola
V. nesbitiana
8909-15 (*V. rupestris* x *V. arizonica)*
8909-19 *(V. rupestris* x *V. arizonica)*
9621-67 *(V. rupestris* x *V. arizonica)*
9621-94 *(V. rupestris* x *V. arizonica*)

Table 2. Combinations of inter-graft stems used for evaluating *Xf* movement. Plants were mechanically inoculated with *Xf* at the base of the susceptible plants (see picture on the right and the bottom). Petioles and leaves from each part of plants were sampled for *Xf* measurement.

Inter-graft stems		
(Susceptible)	(Resistant)	(Susceptible)
8909-19	8909-15	8909-19
Chardonnay	8909-15	Chardonnay
Chardonnay	Haines City	Chardonnay
Thompson Seedless	8909-05	Thompson Seedless
Fiesta	8909-05	Fiesta
9621-94	9621-67	9621-94



Base of the grafted plants. Yellow arrow points to the first graft union. Red arrow indicates the inoculation point. Symptoms in the lower part of the susceptible Chardonnay plant.	Leaves on the shoot coming from the middle part of the inter-stem of the grafted plant (resistant, 9621- 15). No visible symptoms.	Upper part of the grafted plants. The second graft union is shown in yellow arrow. PD symptoms are present on the upper part of the grafted plants (susceptible Chardonnay).

Figure 1. Inter-grafted plant experiment

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FUNDING AGENCIES

Funding for this project was provided by the University of California Pierce's Disease Grant Program.

DEVELOPING TRANSCRIPTIONAL PROFILES AND GENE EXPRESSION ANALYSIS OF GRAPE PLANT RESPONSE TO *XYLELLA FASTIDIOSA*

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Reporting period: The results reported here are for work conducted from November 2003 to September 2004.

ABSTRACT
The goal of the project is to characterize the molecular events in the grape / *Xylella fastidiosa (Xf)* interaction. We used highly resistant and susceptible genotypes from a *Vitis rupestris* x *V. arizonica* population segregating for Pierce's disease (PD) resistance. We developed a functional genomic approach to specifically identify PD-related transcriptional profiles from susceptible and resistant responses. About 5,000 expressed clones have been sequenced and annotated from forward and reverse subtractions of cDNA libraries. These expression profiles derived from the stem, leaf and shoot tissues of resistant and susceptible genotypes throughout the course of disease development provide informative details of molecular events associated with PD. Currently we have identified 63 up/down regulated genes in response to *Xf* infection in both genotypes. To further characterize genes involved in the host-pathogen interaction at different tissues and stages of disease development, we are constructing a set of genes for microarray-based global gene expression analysis. Currently, we are analyzing the first 20 candidate genes using the Taq-Man gene expression assay method. These research efforts will help identify spatial and temporal gene expression involved in the defense response and signaling recognition in PD susceptible and resistant grapes.

INTRODUCTION
The impact of Pierce's disease (PD) on the California grape industry has been exacerbated by the recent introduction and establishment of a more effective vector, *Homalodisca coagulata*, the glassy-winged sharpshooter. Host plant resistance is a critical component of integrated crop management. Traditional breeding has been the main strategy in developing disease/pest resistant plants and is underway in the Walker laboratory. The goal of this breeding program is to develop resistant cultivars, map and develop DNA-based markers for resistance screening, and finally identify resistance genes. Breeding efforts confirm that resistance is inheritable, and molecular mapping has linked DNA markers to *Xylella fastidiosa (Xf)* resistance (see Reports from Walker's grape breeding projects). Once the resistance genes are confirmed, it will be possible to incorporate PD resistance genes from grape species into traditional grape cultivars. However under conventional breeding procedures, several generations will be required to exclude undesirable characteristics from wild species and non-*vinifera* cultivars. In order to speed up resistance gene identification and elucidate the molecular basis of resistance and pathogenicity to *Xf*, we propose here to develop a functional genomic approach for PD research.

Suppression Subtractive Hybridization (SSH) is a powerful tool for comparing two populations of mRNA and elucidates clones of genes that are expressed in one population, but not in the other (e.g. infected vs. control). By using this molecular technique, we are able to selectively enrich these differentially expressed genes, clone and sequence them. This technique has a number of powerful aspects. It is a high efficiency for cloning pathogen-induced genes while removing or reducing constitutively expressed housekeeping genes. 2) The system works particularly well with paired comparisons within a population of segregating siblings. In the case of PD, we used highly resistant and susceptible sibling progenies from a *V. rupestris* x *V. arizonica* cross. Thus, the differences in gene expression patterns between genotypes likely reflect the molecular basis of the resistance and susceptibility responses. 3) The SSH cDNA technique normalizes expressed cDNAs during library construction and therefore significantly increases the chance of cloning genes that are expressed but at very low abundance. This is particularly important because many pathogen-related genes might be expressed at low abundance, and limited to particular tissues or cell types at certain times (Caturla et al., 2002). Some of these genes are less likely to be cloned if a standard EST cloning method is used.

OBJECTIVES
1. Construct twelve tissue-specific reciprocal SSH cDNA libraries from highly resistant and highly susceptible genotypes.
2. Sequence and annotate expressed genes. Identify differentially expressed genes associated with disease development and resistance. Make annotated sequenced genes available to public.
3. Conduct expression gene profile analysis using Microarrary and Taq-Man gene expression technology. Identify genes associated with pathogenicity and genes linked to *Xf* resistance. Elucidate metabolic pathways involved in the pathogenicity and resistance mechanism(s).

RESULTS

Objective 1

RNA Sample Preparation

A pair of highly resistant (#9621-67) and highly susceptible (#9621-94) sibling genotypes selected from segregated population of *Vitis rupestris* x *V. arizonica* were used for this study. Samples were collected from leaf, stem and shoot of infected and non-infected, resistant and susceptible plants at 1, 3, and 5 days after inoculation, followed by 4 collections at 7-day intervals, and then by 4 additional collections at 14-day intervals. The total time from the first inoculation to last sampling was more than 90 days. We used our recently developed a grape RNA extraction protocol for grape stem, leaf and shoot RNA isolation. The average yields of total RNA are 15, 40 and 70 µg/g tissue respectively. mRNAs were further purified from total RNA using the Dynabeads Oligo$(dT)_{25}$ method. About 2-3 µg mRNA was obtained from each sample for constructing cDNA libraries.

cDNA Library Construction

We used our modified version of the CloneTech SSH library construction kit (CLONTECH-Laboratories, 1999) to construct twelve reciprocal SSH cDNA libraries (Table 1). Cloned cDNAs were transformed and quality of each library was evaluated before preparing plasmid DNAs for sequencing work.

Objective 2

Sequencing cDNA Library

Unlike a standard cDNA library, an SSH library selectively clones differentially expressed genes. Depending on the complexity of expression in each expression source, each library usually does not require very deep sequencing. To minimize sequence diminishing return while covering as many genes as possible, 480 (96 x 5) clones were first sequenced from each library. Based on the results of the numbers of contigs and sequence redundancy from each library, more sequences were adjusted to ensure good coverage for all libraries.

Sequence Data Processing

Sequence trace files were scored with cutoff scores of PHRED 20. The FASTA files were trimmed of vector sequences and filtered of non-target sequences such as rRNA and *E. coli*. After contig assembly, BLASTX and BLASTN analyses were performed against the NCBI protein and EST databases, *Arabidopsis* and grape genomic databases. As preliminary annotation, orthologous analysis of *Vitis* expressed genes to *Arabidopsis* is based on the expected values. We grouped the results into three classes as high similarity with E value of $<e^{-30}$ or less, no significant match with E value between $<e^{-6}$ and $<e^{-4}$d and no hit. The "no hit" class is likely to contain *Vitis* specific expressed genes. According to the BLAST reports, we are dividing these contigs into categories according to biological functions such as pathogenesis, disease defense, heat shock, signaling, oxidative metabolism and so on. A possible metabolic role will be assigned to each sequence file.

Objective 3

While we are processing our PD specific transcriptional profile database and designing a set of candidate genes for global gene expression analysis, we identified 63 up/down-regulated transcripts in response to *Xf* infection in both resistant and susceptible genotypes (Table 2). Some of these are putatively involved in pathogenesis, defense response and signal transduction (Figure 1). We used Taq-Man expression analysis method to analyze the first 20 genes. An example of gene expression analysis is presented in Figure 2.

CONCLUSIONS

Characterizing the molecular basis of the grape response to *Xf* is important toward understanding mechanisms of PD resistance and pathogenesis. Expression profiles provide a useful framework for the next step of expression analysis that will help to further dissect genes underlying metabolic pathways involved PD responses.

Table 1. Forward and reverse SSH cDNA library construction for both resistant and susceptible genotypes.

Genotypes	Resistant or susceptible genotype		
Tissues	Leaf	Stem	shoot
Forward subtraction	Infected ← health	Infected ← health	Infected ← health
Reverser subtraction	Infected → health	Infected → health	Infected → health

Table 2. Summary of up-regulated and down-regulated transcripts between resistant and susceptible genotypes among three tissues following of *Xf* infection

Genotypes	Tissue	Up Regulated	Down regulated
Resistant (9621-67)	Stem	8	6
	Leaf	1	2
	Shoot	16	3
Susceptible (9621-94)	Stem	8	5
	Leaf	3	2
	Shoot	7	2

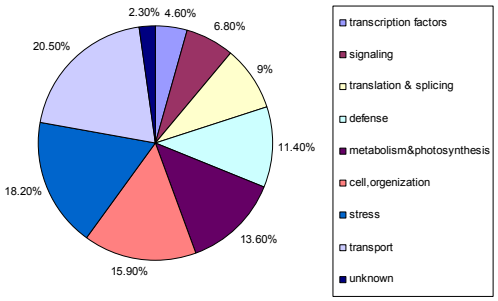

Figure 1. Functional category of putative genes of among 63 differentially expressed transcripts.

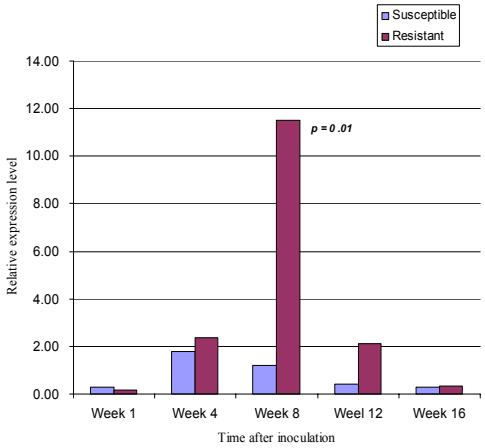

Figure 2. Taq-Man gene expression analysis was used to analyze expression during PD development. Here is an example of the putative pathogenesis-related gene, which increased more than 10 times the transcriptional levels in the 8th week after inoculation in the susceptible genotype (9621-94) as compared to the resistant genotype (9621-67).

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FUNDING AGENCIES

Funding for this project was provided by the CDFA Pierce's Disease and Glassy-winged Sharpshooter Board.

CORRELATION BETWEEN RESISTANCE TO PIERCE'S DISEASE AND *XYLELLA* STRAIN VIRULENCE USING PARTIALLY PURIFIED CULTURE FILTRATE

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Reporting Period: The results reported here are from work conducted from March 2003 to September 2004.

ABSTRACT
Previous research at the FAMU Center for Viticulture suggested that cells of a virulent strain of *Xylella fastidiosa* (*Xf*) may produce toxic compounds that could be used to determine varietal susceptibility to Pierce's disease (PD) in grapes. I n the experiments reported here, when grape leaves were challenged with partially purified culture filtrate of *Xf* with different levels of virulence, positive correlations between the degree of leaf necrosis and (1) the virulence of the *Xf* strain and (2) the level of PD resistance were observed.

INTRODUCTION
Pierce's disease (PD), a lethal disease of grapevine, is caused by the bacterium *Xylella fastidiosa* (*Xf*) (Proteobacteria: Xanthomonadales) and is spread by leafhoppers known as sharpshooters. *Xylella fastidiosa* is native to the southeastern U.S., where it reproduces in ornamentals such as crape myrtle, eucalyptus, and hibiscus, but also in various crop plants including citrus, avocado and grapes (Blua et al. 1999). In Florida and other southeastern States, the abundance of *Xf* and vectors such as the glassy-winged sharpshooter (*Homalodisca coagulata*) has precluded commercial production of European grape varieties. The first evidence of PD infection usually is a drying or "scorching" of leaves. Typically, the leaves dry progressively over a period of days to weeks, showing a series of concentric zones of discolored and dead tissue. Vines develop symptoms as the bacteria multiply and begin to block the water conducting system and reduce the flow of water to affected leaves. However, Hopkins (1983) reported that only about 40% of the xylem vessels of infected plants have bacterial occlusions and plants with this percentage of non-functioning vessels typically do not show symptoms of water stress. The PD bacterium also has been reported to produce a phytotoxin or phytotoxins that may damage plant tissues and play an important role in disease initiation and development (Lee 1982).

OBJECTIVES
1. Determine whether partially purified culture filtrate from virulent, weakly virulent and avirulent strains of *Xf* would produce different levels of necrosis when applied to leaves of a given variety of grape.
2. Determine whether partially purified culture filtrate from a given strain of *Xf* would produce different levels of necrosis when applied to leaves from susceptible, tolerant and resistant varieties of grape.

RESULTS AND CONCLUSIONS
Cultures of virulent (PD002), weakly virulent (PD91-2) and avirulent (PD F1) strains of *Xf* were centrifuged to remove cells. The supernatant was filtered and then extracted with ethyl acetate, and the eluate was evaporated to dryness. The powder was then reconstituted in distilled water and applied to the surface of detached leaves of different grape varieties that had been wounded with a sharp needle. After 48 h, the leaves were scored based on the percentage of the leaf surface with necrotic lesions (Table 1).

In general, the mean percentage of leaf necrosis was greater when leaves were challenged with partially purified culture filtrate (PPCF) from the more virulent strains of *Xf*. For example, the leaf necrosis rating for 'Chardonnay', a highly PD susceptible variety of *V. vinifera* grape, was 1.5 for the virulent strain of *Xf*, 0.9 for the weakly virulent strain and 0.3 for the

avirulent strain. The leaf necrosis ratings for Black Beauty, a PD tolerant variety of muscadine grape, were 0.7, 0.4 and 0.1 when challenged with PPCF from the virulent, weakly virulent and avirulent strains of *Xf*, respectively.

In addition, leaves from susceptible varieties of grape generally produced greater levels of necrosis than did leaves from tolerant and resistant varieties. For example, the mean percentage of leaf necrosis for 'Chardonnay', 'Blanc du Bois' (a PD tolerant Florida hybrid bunch grape), Alachua and Noble (PD resistant muscadine grapes) were 1.5, 1.0, 0.6 and 0.0, respectively, when challenged with the PPCF from the virulent strain of *Xf*. Similar and consistent trends also were observed when using PPCF from the weakly virulent and avirulent strains of *Xf*, but as mentioned before, the leaf necrosis ratings were lower, which resulted in less overall differences between susceptible and resistant varieties.

These results suggest that *Xf* may produce extra cellular "toxin(s)" that could cause necrotic lesions when applied to grape leaves and that might have potential in screening grape germplasm and hybrids for PD resistance. The "toxins" extracted from the culture filtrate of more virulent strains of *Xf* produced more necrosis than did the "toxins" from less virulent strains. Leaves from susceptible varieties of grape also reacted more strongly to these "toxins" than did the leaves from resistant grape varieties. At this time the nature of the "toxin(s)" is not known, nor is it known whether the different strains of *Xf* produce different quantities or types of these "toxins". Future studies will attempt to answer these questions and expand the number of PD susceptible and resistant grape varieties and *Xf* strains evaluated with this test.

A

B

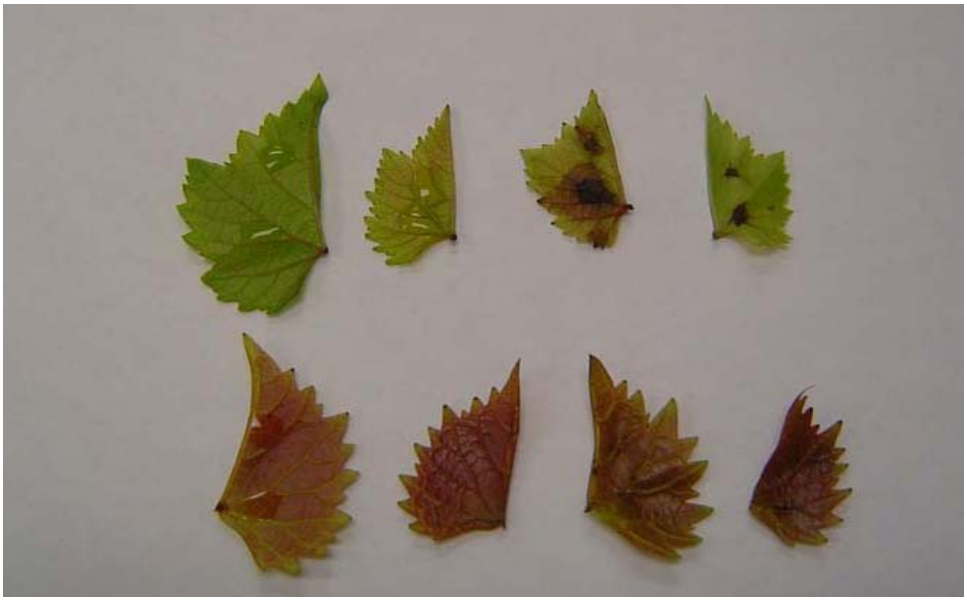

Figure 1. An example of the type of symptoms caused by *Xf* culture filtrate in young 'Chardonnay' (A, PD susceptible) and 'Noble' (B, PD resistant) grape leaves. Lanes 1 and 2 = control leaves treated with distilled water, lane 3 = leaves treated with undiluted culture filtrate from a virulent strain of *Xf* PD002, and 4 = leaves treated with diluted (1:2 vol/vol) culture filtrate of *Xf* PD002. Incubation time was 48 h.

Table 1. Response of grape leaves to partially purified culture filtrate from virulent (PD002), weakly virulent (PD91-2), and avirulent (PD-F1) strains of *Xf* as measured by the amount of necrosis produced. Leaf necrosis ratings were: 0 = no necrotic lesions; 1 = 25% or less of the leaf surface with necrotic lesions; 2 = 26-50% necrosis; 3 = 51-75% necrosis; 4 = 76-100% necrosis. The level of PD resistance: S = Susceptible, T = Tolerant and R = Resistant.

Virulent Strain (PD002)

	Leaf Necrosis Rating by Replicate											
Grape Variety	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**Mean**	**Control**
Chard. (S)	1	1	1	1	2	2	1	2	2	2	1.5	0
Blc. Bois (T)	1	1	1	1	1	1	0	1	1	2	1.0	0
Carlos (T)	0	1	0	1	1	1	2	0	1	1	0.8	0
Bl. Beauty(R)	1	1	0	1	1	1	1	1	0	0	0.7	0
Alachua (R)	1	0	1	0	1	0	1	1	0	1	0.6	0
Fry (R)	1	0	0	1	1	0	1	1	0	0	0.5	0
Noble (R)	0	0	0	0	0	0	0	0	0	0	0.0	0

Weakly Virulent Strain (PD91-2)

	Leaf Necrosis Rating by Replicate											
Grape Variety	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**Mean**	**Control**
Chard. (S)	1	1	1	0	0	1	1	2	1	1	0.9	0
Blc. Bois (T)	1	0	0	0	1	1	0	1	1	0	0.5	0
Carlos (T)	0	0	0	1	0	1	0	0	1	0	0.3	0
Bl. Beauty(R)	0	0	1	1	0	0	1	0	1	0	0.4	0
Alachua (R)	0	1	0	0	0	1	0	0	1	0	0.3	0
Fry (R)	0	0	1	1	0	0	0	0	0	0	0.2	0
Noble (R)	0	0	0	0	0	0	0	0	0	0	0.0	0

Avirulent Strain (PD-F1)

	Leaf Necrosis Rating by Replicate											
Grape Variety	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**Mean**	**Control**
Chard. (S)	0	1	0	0	0	0	1	0	1	0	0.3	0
Blc. Bois (T)	0	0	0	0	0	1	0	0	0	0	0.1	0
Carlos (T)	0	0	0	0	0	0	0	0	0	0	0.0	0
Bl. Beauty(R)	0	0	0	0	0	0	0	0	1	0	0.1	0
Alachua (R)	0	0	0	0	0	0	0	0	0	0	0.0	0
Fry (R)	0	0	0	0	0	0	0	0	0	0	0.0	0
Noble (R)	0	0	0	0	0	0	0	0	0	0	0.0	0

Abbreviations: Chard = Chardonnay, Blc. Bois = Blanc du Bois, Bl. Beauty = Black Beauty.

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FUNDING AGENCIES

Funding for this project was provided by the USDA Animal and Plant Health Inspection Service, the USDA Cooperative State Research, Education, and Extension Service, and the Florida Viticulture Advisory Council.

TOWARDS IDENTIFYING PIERCE'S DISEASE RESISTANT GENES FROM A NATIVE AMERICAN GRAPE SPECIES (*VITIS SHUTTLEWORTHII)* – A GENOMICS APPROACH

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ABSTRACT

INTRODUCTION
There are over 160,000 grape ESTs in the public data bases and the vast majority of these ESTs were generated from the European grape varieties (*Vitis vinifera*). However, the European grapes are highly susceptible to the Pierce's disease and they are not necessary possessing all the genes required for providing a full protection against the GWSS and *Xf* attack. On the other hand, PD resistant sources exist in some native North American grape species, particularly those species originated in the southeast United States. For example, *Vitis shuttleworthii*, a species originated from the southeast United States, is considered to be one of the most PD resistant grape, which has long been used for developing PD resistant grape varieties for the deep south - a most severe PD infected area. We therefore propose to search for PD resistant genes from the *Vitis shuttleworthii* grape.

The Viticulture Center at Florida A&M University and the USDA-ARS Horticultural Laboratory at Fort Pierce (Florida) jointly initiated a grape EST project from the native American grape -*Vitis shuttleworthii*, aiming to identify and isolate grape disease resistant genes including the Pierce's disease resistant genes. We have sequenced 30,000 ESTs, and have several on-going experiments for expression analysis and marker development for identifying the PD resistant genes.

OBJECTIVES
The objectives of this research are to identify/isolate PD resistant genes from *Vitis shuttleworthii* grapes and develop EST derived molecular markers for PD resistance. Specifically, the project is gearing towards to: 1) discover genes for PD resistance from *Vitis shuttleworthii* grapes; 2) conduct comparative genomics analysis between *V. shuttleworthii, V. vinifera* grapes and other plant species; 3) develop SSR and SNP markers for PD resistance, which will be used for accelerating the development of PD resistant grape varieties.

RESULTS AND CONCLUSIONS
We have sequenced 30,000 ESTs from a clone of *V. shuttleworthii* grape. Blasting analysis revealed that 13% of the *V. shuttleworthii* ESTs are unique when compared to the existing *Vitis vinifera* NCBI databases, and 3% of the ESTs did not find any homologous sequences among all plant ESTs reported in NCBI. Overall, approximately 7% of ESTs were related to disease / pest defense or stress tolerance genes, and it is obvious that these genes are abundant in the *V. shuttleworthii* grape (Table 1, Table 2).

Table 1. Comparison of transcription factor (TF) families in grape (*V. shuttleworthii, V. vinifera*), *Arabidopsis* and *R*ice

V. vinifera	Arabidopis	Rice
124	190	156
69	144	143
114	105	125
67	82	71
131	72	83
34	36	21
31	28	8
121	81	75
10	6	5

Table 2. Comparison of disease resistant gene (R-gene) families in grape (*V. shuttleworthii, V. vinifera*) and *Arabidopsis*

R-gene Class	Number in V. shuttleworthii	Number in V. vinifera	Number in Arabidopis
TIR-NBS-LRR	11	64	85
CC-NBS-LRR	9	51	41
NBS-LRR	9	64	10
TIR-NBS	3	19	17
CC-NBS	5	30	4
TIR	18	82	36

A series of experiments are being conducted to identify and isolate PD resistant genes through gene expression profiling analysis by using DNA microarrays. Specifically, a comparative analysis of transcriptional profiles of 1) unchallenged *V. shuttleworthii* grapes (control), *Xf* challenged *V. shuttlewothii* grapes (samples will be collected on different timeframes after infection).

For marker development, we are developing SNP and SSR markers from our *V. shuttleworhtii* sequence data set and the *V. vinifera* ESTs in the public domain. Aligned sequences will be mined for Single Nucleotide Polymorphism. A preliminary screening of the SNP and SSR marker from the 12,056 *V. shuttleworthii* ESTs indicated that the SNP and SSR markers are abundant in *V. shuttleworthii* grapes, and around 800 candidate SSR and SNP sites have already been identified. Table 3 shows the distribution of the di-, tri-, and tetra- SSRs from *Vitis shuttleworthii* ESTs, and Table 4 shows the abundant SSRs motifs from *Vitis shuttleworthii* ESTs. We have designed and synthesized the PCR primer pairs using computer software such as Primer3 to flank the SSR loci (partially shown in Table 5). Verification of these primers with PCR amplification on selective grape DNA templates is under way.

Table 3. Distribution of EST derived SSRs from *Vitis shuttleworthii*

Number of ESTs	Number of SSR-ESTs	Motifs		
		di-	tri-	tetra-
10,995	401(3.651[1])	82(20.32[2])	306(76.5)	13(3.2)

[1.] SSR-EST percentage in total EST
[2.] di-nucleotide motif percentage in SSR-EST.

Table 4. Distribution of the abundant (>5) SSR-ESTs among the *V. shuttleworthii* EST data set.

SSR Motif	Number of ESTs
GA/CT	36
AT/TA	13
CAA/GTT	90
ACC/TGG	34
TCT/AGA	19
CAG/GTC	15
AAG/TTC	14
CAC/GTC	14
CTT/GAA	13
CCA/GGT	12
CCT/GGA	12
TGA/ACT	9
TCC/AGG	8
CAT/GTA	7
GAT/CTA	6
TGC/ACG	6
CTC/GAG	5
Total	313

Table 5. A selective set of SSR primer pairs from the *Vitis shuttleworthii* ESTs

Repeat	*Left Sequence*	*Right Sequence*	*Product Size*
GTCGTCGTCGTCGTCGTCGTC	TACAAGAGCCAAGAGGGATT	GGATAACGAAGGAGACAGAGT	245
AGCAGCAGCAGC	AGGGAGATGACAAAGATGAAG	CCAAACACCGTAGGAGAGA	367
AACAACAACAACAAC	AATAATAAGAAGGAGATGCGG	GTTGTGGTGGTCGTGAAG	367
AGCAGCAGCAGC	CAGAGTGTCAGCACAGCA	GCGTTTCTCAAGGTTCTACTT	368
AACAACAACAACAAC	TGACTGGCATACTGATTTACC	CCCAATGAACTACCTTTACCT	368
CGGCGGCGGCGGCGGCGG	ACCCAATGAACTACCTTTACC	AGGAACAAGACAAACAATACACT	113
CCTCCTCCTCCTCCTCCTCCT	TTTATCCCAACAATCAGG	CTTTCACAGCAGAAGAGTT	226
CCTCCTCCTCCTCCTCCTCCT	GCCTTGGACCGAACTATC	CTAAGAAACACCATTCATCAG	226
GAGAGAGAGAGAGAGAGAGAGA	CGACCTAAGAAACACCATTC	CCTTGGACCGAACTATCTG	292
ACCACCACCACCACCACC	CGCATCAGAAGTCATCAAC	ACCCTCACTCTCACACTCAC	238
TCCTCCTCCTCCTCCTCC	ACGGAAGAAGAGAAGAAAGAG	ATCCACCGAAACAAACTTAC	133
AGAAGAAGAAGAAGAAGA	ACAAAGCAGGTAAGTAGCAAA	AAGACGGAAGAAGAGAAGAAA	233
TCTCTCTCTCTC	GTGATTGTTACCGACCTTGA	ATTCCCTTCTTCTCCTTTACC	195
TCTCTCTCTCTC	CCTCGGAAACAAACTTACA	CGAAGAAGAGAAGAAAGAGAAA	195
TGATGATGATGATGATGAGGATGATGA	AAGACCGAAGAAGAGAAGAAA	TAATACCGTGGAAATCACAAA	281
ACCTACCTACCTACCTACCT	TTACCCGACACTGGACAC	ACTTACCACCGAGATGAGG	266

After the potential SNP-EST and SSR-EST are verified, PD segregating populations will be used for marker development. Several populations derived from the hybridization of Native American species/hybrids and *V. vinifera* grapes will be candidates for this purpose. For example, a 183-seedling population of N18-6 x 'Cabernet Sauvignon' has been evaluated for PD resistance for several years in our vineyard. **'**N18-6' is a breeding line highly resistant to PD while 'Cabernet Sauvignon' is the best known wine grape variety highly susceptible to PD. Segregating analysis revealed that three dominant genes provide full resistance to the Pierce's disease**.**

FUNDING AGENCIES
Funding for this project was provided by the USDA Cooperative State Research, Education, and Extension Service, and the FAMU-ARS Science Center.

FIELD EVALUATION OF GRAPE ROOTSTOCK RESPONSE TO NATURAL INFECTION BY PIERCE'S DISEASE

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Reporting Period: The results reported here are for work conducted from November 2, 2003 to October 31, 2004.

ABSTRACT
To understand the adaptation of grape rootstocks commonly used in major grape production areas worldwide to Florida, where Pierce's disease (PD) is the primary limiting factor in grape production, ten important grape rootstocks were cultivated at the experimental vineyard, Florida A&M University, Tallahassee, Florida. Disease resistance and symptoms and growing performance were evaluated. PD symptoms were scored in September and October 2002, 2003, and 2004, with leaf symptoms the basis of scoring. None of the grape rootstocks was completely resistant to PD and the severity of PD varied with rootstock cultivar. St George and Ramsey showed least PD symptoms. Freedom and 44-53 succumbed to PD by the 2004 rating period; of the surviving rootstocks, 3309C had the highest PD score. Overall vine survival, evaluated in 2002, 2003, and 2004, varied among the rootstocks. Based on the performance of ungrafted vines, St George and Ramsey are the most suitable rootstocks in this north Florida environment, where natural infection by PD is very high and vectors and inoculum are abundant.

INTRODUCTION
Rootstocks are used widely in viticulture to provide resistance against soil pests and pathogens and improve scion performance. Choice of rootstock depends on pest populations, soil, and growing conditions. The grape rootstocks in common use world wide are deployed primarily to provide phylloxera and nematode protection (Bouquet 1980, Einset and Pratt 1975, Winkler et al 1974). In contrast, Pierce's disease (PD), caused by gram-negative bacterium *Xylella fastiosa* (*Xf*), is the primary limiting factor of growing *Euvitis* grape in the southeast United States (Lu and Ren 2002, Chen et al 2001). Pierce (1905) reported that rootstock variety affected expression of "California vine disease" (now known as Pierce's disease) in grape. Grape rootstock trials in Mississippi showed a large effect of rootstock trial on vine longevity in a region recognized for high Pierce's disease pressure (Loomis 1965, 1952, Magoon and Magness 1937). In humid and hot regions of the United States, such as Florida, bunch grapes often are highly susceptible to pests and diseases (Olien and Hegwood 1990). When the Florida hybrid bunch grape cultivar Blanc du Bois was grafted on to muscadine, which is relatively tolerant or resistant to the bunch grape pests and diseases common in North America, the scion showed a reduction in both PD and anthracnose symptoms and fruiting improved (Ren and Lu 2002). Growing conditions in Florida are harsh-a successful rootstock for grape industry in that area must be tolerant to PD and adapted to the environment. Evaluation of rootstock performance and survival in Florida would provide useful information on rootstocks performance for humid tropical and subtropical environments, especially where PD is prevalent. Greenhouse screening has been used to investigate the PD resistance, tolerance, and susceptibility of grape cultivars. However, field screening is more applicable, since conditions closely match those in a commercial vineyard. When relying on natural infection in the vineyard, there is no need to inoculate vines or maintain colonies of *Xf* or insect vectors. Field screening is cheap, requires no specialized equipment and can be accomplished quickly, with symptom expression being used as the main criterion. Northern Florida is an ideal test environment due to heavy PD pressure, with abundant vectors, including glassy-winged sharpshooter, and inoculum, in contrast to many other locations, especially California, which demonstrate substantial cycling of PD incidence.

OBJECTIVES
1. Evaluate the response of grape rootstocks to natural field infection by Pierce's disease.

RESULTS AND CONCLUSIONS
Ten grape rootstocks (five replicates of two vines each, ten vines total per rootstock cultivar) were planted in the spring of 2001. Vines were bilaterally cordon trained and spur pruned. Pierce's disease (PD) symptoms were scored in 2002, 2003 and 2004, with symptoms on leaves assessed in a numerical scale from 0 to 5. For PD, 0 represented no symptoms, 1 = minor symptoms up to 15% of leaves with marginal necrosis (MN), 2 = 15-30% of leaves with MN, 3 = 30-50% of leaves with MN, 4 = 50-75% of leaves with MN, 5 = over 75% of leaves with MN or vine dead. Vine vigor was surveyed later fall in 2002. The annual shoot and node growth was recorded from ten randomly sampled shoots per plant, and shoot diameter was taken in the middle of 4th node. Node length was calculated with total node numbers and the length of each shoot. Twenty (4 x 5) random shoots were investigated for shoot death rate from each vine: 5 shoots in each canopy quadrant area divided by the main trunk and trellis wire. A shoot was considered as dead if more than half of the shoot had died. Trunk diameters were measured 50 cm above the ground in fall 2003.

All rootstock vines developed PD symptoms, although the severity varied. The least severe PD scores were seen on Ramsey and St George, with average PD scores of 1.1 and 1.4 in 2002, 1.0 and 1.7 in 2003, and 1.2 and 0.9 in 2004, respectively (Table 1). The consistently low PD scores on these varieties over several years demonstrate that Ramsey and St. George are reliably resistant or tolerant of PD in north Florida.

Freedom (3.7 – 5.0 score in 2002-2003) and 44-53 (2.6 – 2.3 score in 2002-2003) did not survive through the rating period of 2004. That Freedom succumbed to PD is not surprising—this rootstock showed the worst PD symptoms of all the rootstocks in the trial in the previous two years of observations. The 44-53 showed severe PD symptoms in 2002 and 2003, but typically its symptoms were not as severe as those on O39-16 and 3309C, so it was surprising that this rootstock succumbed while O39-16 and 3309C remain in the trial.

Of the surviving rootstocks, 3309C (3.0) and 5BB (2.9) had the most severe PD symptoms in 2004. The 3309C has consistently shown heavy PD symptoms and most of the vines of this rootstock have died (Table 3). The slightly less severe average PD score for 3309C probably reflects the survivorship of this vine (heavier symptoms being related to lower survivorship). Although 5BB showed excellent survivorship in earlier years of the study, it is now beginning to develop PD symptoms. The 5C, 110R, and 101-14 showed moderate PD symptoms over the three year period (Table 1). O39-16 symptoms in 2004 were less severe than in earlier years, when it was among the most symptomatic rootstocks; however, symptom severity overall was lower in 2004.

After four growing seasons in Florida's heavy PD pressure, environment, the survival rate was very different among the rootstocks (Table 2). Only Ramsey shows 100% survival. All Freedom and 44-53 vines have been killed by PD and only one of ten 3309C vines remains alive. Vines greatly deteriorated in the third growing season; from 2002 to 2003, the vine losses of Freedom, 44-53 and 3309C were 87%, 70%, and 50%, respectively. There was less change overall in vine survival from 2003 to 2004. Although Freedom and 44-53 completed their precipitous decline, other varieties may be reaching a "steady state" of vine survival, with diminishing losses to PD. The 110R, 5C, and 101-14, noted for their moderate PD symptoms, have survival rates of at least 80%.

Fishleder (2000) examined the response of grape rootstocks to PD in a greenhouse. In contrast to this study, Fishleder inoculated vines with *Xf*. The results from this study largely coincide with and confirm Fishleder's findings. In particular, both this research and Fishleder's work found St George to show only minor PD symptoms; O39-16, 5C, 5BB, 110R were intermediate in symptom development; and 3309C and Freedom showed severe PD symptoms. However, our results contradict Fishleder's regarding Ramsey. While we observed only low levels of PD symptoms in Ramsey, Fishleder found Ramsey to be one of the most symptomatic of rootstocks tested. What accounts for this disparity in observation? It is possible that the *Xf* strain that Fishleder cultured and used to inoculate the vines growing in the greenhouse was substantially different in pathogenicity or host specificity from the naturally occurring *Xf* prevalent at Tallahassee, Florida. Another possibility is that while the *Xf* populations in the respective studies do not differ in pathogenicity or host specificity, the direct inoculation through pin prick employed by Fishleder is more difficult for the plant to resist than the natural inoculation by insect vectors that is thought to have occurred in the vineyard.

Rootstock performance in north Florida primarily is a factor of PD response. Cultivars differed in their performance and some were markedly superior-these should be further investigated for their influence on scions. Specifically we suggest Ramsey and St George for additional study. These rootstocks survive well under natural inoculation conditions in north Florida. The evaluation of rootstock cultivars in PD limited viticultural regions is important-much PD management research is focusing on augmenting PD resistance and or tolerance in scions, but rootstocks are a critical component of viticulture. As demonstrated here, several rootstocks have substantial levels of PD resistance that should permit their cultivation in PD prone regions, allowing concentration of effort on scion improvement. Additionally, testing the PD response of ungrafted rootstocks indicates the potential for rootstock varieties to be cultivated as nursery mother vines in PD prone regions. Rootstocks identified as resistant or tolerant to PD could be genetic resources for breeding improved PD resistant scion varieties, as in the case of MidSouth and MissBlue, which have PD resistant rootstocks as parents (DeGrasset and Dog Ridge, respectively). PD resistant rootstocks might be necessary for the cultivation of PD tolerant scion varieties if *Xf* spreads to the root system.

Field evaluation of PD resistance in Florida is easy due to high PD pressure resulting from high populations of vectors and bacteria in the area and should be continued as a technique to test PD management strategies and screen plant material.

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Table 1. PD symptom scores of the ten grape rootstocks during the second, third, and fourth growing seasons.

Rootstock	PD score		
	2002	2003	2004
O39-16	3.1bc	3.8b	2.3
101-14	2.2d	2.4c	1.9
110R	2.2d	1.8cd	2.3
3309C	3.6b	4.2ab	3.0
44-53	2.6cd	2.3c	---
5BB	2.7cd	1.6cd	2.9
5C	2.2d	1.9cd	2.1
Freedom	3.7b	5.0a	---
Ramsey	1.1e	1.0d	1.2
St. George	1.4e	1.7cd	0.9

Table 2. Vine survival of the ten grape rootstocks after four growing seasons.

Rootstock	Number of living vines				Survival %
	2001	2002	2003	2004	2004
O39-16	9	9	6	6	67
101-14	10	10	10	9	90
110R	10	10	9	8	80
3309C	10	10	5	1	10
44-53	10	10	3	0	0
5BB	10	10	10	7	70
5C	10	10	9	9	90
Freedom	10	8	1	0	0
Ramsey	8	8	8	8	100
St. George	10	9	9	7	70

FUNDING AGENCIES
Funding for this project was provided by the University of California Pierce's Disease Grant Program. Special thanks to California Grapevine Nursery for supplying the grapevines used in this experiment.

MECHANISMS OF PIERCE'S DISEASE TRANSMISSION IN GRAPEVINES: THE XYLEM PATHWAYS AND MOVEMENT OF *XYLELLA FASTIDIOSA*. PROGRESS REPORT NUMBER ONE: COMPARISON WITH SYMPTOMS OF WATER DEFICIT AND THE IMPACT OF WATER STRESS

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Reporting period: The results reported here are from work conducted from October 2003 to September 2004.

ABSTRACT
The pathology of diseases such as Pierce's disease (PD) of grapevine (*Vitis vinifera* L.) that are caused by the xylem-limited bacteria *Xylella fastidiosa (Xf)* is widely attributed to vessel occlusion and subsequent water deficits. Grapevines (*Vitis vinifera* L. 'Chardonnay') were exposed to water deficits, stem inoculation with *Xf*, and combinations of both to evaluate whether symptoms of PD were a consequence of water deficits. When vines were inoculated with *Xf* and exposed to water deficits, more extensive PD symptoms developed throughout the plant than when + *Xf* vines were well-watered. However, vines infected with *Xf* exhibited symptoms unique to PD that included inhibited periderm development in stems (green islands), leaf blade separation from the petiole (matchsticks), and irregular leaf scorch. Vines exposed to water deficits and not *Xf*, displayed accelerated periderm development, basal leaf abscission at the stem/petiole junction, and uniform leaf chlorosis. Water deficits induced the development of an abscission zone, but PD did not. Pierce's disease symptoms could not be produced with any of several water deficit treatments, including severing all but one secondary vein near the leaf tip. The results indicate that factors other than water deficits are involved producing the symptoms of PD. We conclude that the widely accepted hypothesis that PD-infected plants develop water deficits that cause green islands, matchsticks, localized leaf scorch, and eventual death of vines should be reevaluated.

INTRODUCTION
The overwhelming consensus among researchers is that the fatal nature of PD is a result of the *Xf* bacteria becoming systemic and blockage occurring in xylem vessels (due to bacterial accumulation, tyloses, gums, and/or emboli), causing water transport to become progressively impaired until the plant is no longer able to function (Goodwin *et al.*1988a, b; McElrone *et al.* 2001, 2003; Newman *et al.* 2003, 2004; California Agricultural Research Priorities 2004). Indeed, Pierce's disease has become nearly synonymous with plant water deficit. This view is largely based on correlative evidence. Hopkins (1988) showed a strong association between reduced water conductance in stems of citrus seedlings and *Xf*-caused disease symptoms. Low leaf water potential and turgor, impaired hydraulic conductance, and higher stomatal resistance were correlated with PD symptoms in grapevines (Goodwin *et al.* 1988a). While reduced leaf water potential, stomatal conductance and stem hydraulic conductivity are characteristic of water deficit, it should be noted that these same features also occur in flooded plants (Kramer & Boyer 1995), so correlations are not necessarily indicative of causality.

From our recent work we observed that, although PD symptoms have been attributed to water deficit, the visual symptoms of PD did not appear to be the same as those resulting from water deficit alone. In grapevine, typical visual symptoms of PD are "green islands," patchy or marginal leaf necrosis (often called leaf scorch), and "matchsticks" (petioles that remain attached to the stem after the laminae have fallen off) (Purcell 1986; Goheen & Hopkins 1988, 1989; Stevenson *et al.* 2004). These symptoms are not characteristic of water deficit symptoms in grapevines (Okamoto *et al.* 2004). In addition, the diagnostic symptoms of PD have never been observed in healthy grapevines exposed to water deficits, nor have they ever been reported to develop as a consequence of water deficits.

Interestingly, citrus trees already infected with *Xf* and subjected to drought displayed accelerated symptom development of citrus variegated chlorosis (Gomes *et al.* 2003). Extended water deficit also increased the severity of Pierce's disease in the woody liana, Virginia creeper (McElrone *et al.* 2001, 2003). Thus, extended water deficit (such as drought) may exacerbate the development of PD symptoms in grapevine as well. However, there are no reports describing the effects of water deficit on *Xf*-infected grapevines, nor has there been a detailed comparison of water deficit and PD symptoms. If the visual symptoms of PD are not, in fact, a result of water deficit, then studies relying on the assumption that water stress is the

ultimate killer of plants suffering from PD may result in misleading information and add years to finding solutions to the PD problem. Therefore, it is important that it be determined which PD symptoms, if any, are a result of water stress, and what role water shortage actually plays in symptom development and vine death.

OBJECTIVES

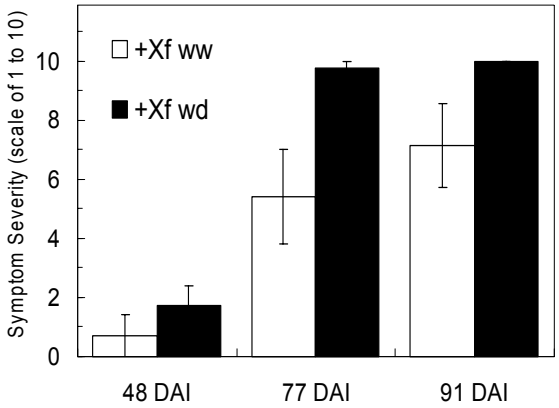
1. Evaluate the impact of vine water status on the development of the visual symptoms of PD.
2. Determine whether visual PD symptoms are a direct result of water deficits.

RESULTS

Objective 1

In the field, extended water deficit exacerbates citrus varigated chlorosis in citrus (Gomes *et al.* 2003) and PD in Virginia creeper (McElrone *et al.* 2001, 2003). Thus, it was not surprising that subjecting potted grapevines to extended water deficit also resulted in a faster and more extensive onset of PD symptoms (barring green islands) than in well-watered *Xf*-infected (+*Xf*) vines. The first clear indications of leaf scorch were seen 48 DAI. Water-stressed +*Xf* vines developed more symptomatic leaves with severe symptoms than well-watered +*Xf* vines (Fig. 1). Interestingly, the leaf scorch and matchstick symptoms in the well-watered +*Xf* plants had the same visual characteristics as in the +*Xf* water-stressed plants. There was no significant difference between well-watered +*Xf* and healthy (-*Xf*) vines in stomatal conductance (0.86 ± 0.09 & 0.69 ± 0.06 cm s^{-1}), transpiration (6.53 ± 0.83 & 5.66 ± 0.83 µg cm^{-2} s^{-1}), and leaf water potentials (-0.60 ± 0.05 & -0.73 ± 0.11 MPa, respectively). Likewise, these parameters were equivalent for water-deficit +*Xf* and –*Xf* vines (0.28 ± 0.04 & 0.34 ± 0.05 cm s^{-1}, 2.41 ± 0.31 & 2.86 ± .39 µg cm^{-2} s^{-1}, -1.07 ± 0.05 & -1.28 ± 0.13 MPa, respectively).

Figure 1. Symptom development during whole plant water deficit and well-watered conditions. Severity of Pierce's disease symptoms in Chardonnay grapevines. Vines infected with *X. fastidiosa* were well-watered (+*Xf* ww; white bars) or subjected to water deficit (+*Xf* wd, black bars). On a scale from 1 to 10, 1 indicates mild symptoms, 10, the most severe PD symptoms. Non-infected values are zero due to the lack of PD symptoms and are not shown.



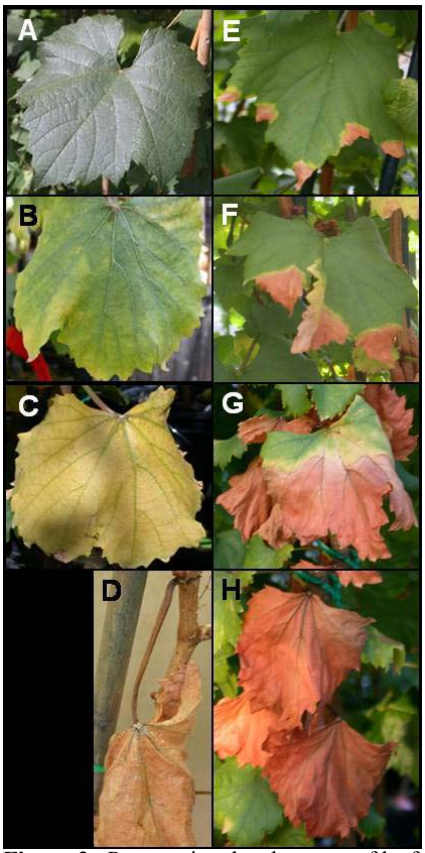


Figure 2. Progressive development of leaf symptoms of non-infected water-stressed Chardonnay leaves (A-D), and Chardonnay with Pierce's disease (E-H).

Objective 2

The results revealed that visual symptoms of Pierce's disease in grapevine are qualitatively and quantitatively different than those of extended water deficit. Regardless of water status, +*Xf* plants displayed symptoms unique to PD. In general, PD symptoms masked water-deficit symptoms. The PD symptoms manifested in laminae, petioles and stems often revealed an interaction between plant and bacteria in which plant responses to *Xf*-infection seemed to be either elicited or suppressed by the bacteria.

Comparison of Visual Symptoms of Water Deficit and PD

To determine whether PD symptoms are a direct result of water deficit, the visual characteristics of well-watered and water-stressed grapevines inoculated with *Xylella* (+*Xf*) or water (–*Xf*) were evaluated. Leaves of well-watered –*Xf* grapevines remained green and healthy throughout the course of the experiments (Fig. 2a). Water-stressed –*Xf* vines gradually developed leaf chlorosis in a fairly uniform pattern over the entire leaf lamina (Fig. 2b-c), with the veins staying green until leaves became necrotic. Leaves remained attached to the stem even after the leaves were apparently dead (Fig. 2d). In contrast, the first PD symptom to appear was leaf scorch. Leaf scorch symptoms started with chlorosis at the margins of the leaves and moved towards the petiole in patches such that sections of necrosis were bordered by slim regions of chlorosis (Fig. 2e-f). As symptoms progressed, laminae of +*Xf* vines became completely necrotic, while the petioles remained green (Fig. 2g-h). Eventually laminae fell from the petioles to form "matchsticks."

In –*Xf* water-stressed plants, two sites of constriction and necrosis developed on petioles, one at the stem/petiole junction (the basal end of the petiole) and the other at the petiole/lamina junction (the distal end of the petiole). At the basal end of the

petiole, a true abscission zone formed. At the distal end of the petiole where the lamina is attached, the tissue constricted and concurrently became necrotic. Observations at the cellular level suggest that the constriction and necrosis at this junction is not an actual abscission zone (Stevenson *et al.* 2004). Neither the abscission zone at the stem/petiole junction nor the fracture zone at the petiole/lamina junction developed until the lamina was severely chlorotic. In +*Xf* vines, a fracture zone also occurred at the petiole/lamina junction. Comparisons of the anatomy of the fracture zone at the petiole/lamina junction of +*Xf* vines and –*Xf* water-stressed vines showed that these fracture zones were identical. However, abscission zones did not develop at the stem/petiole junction of either well-watered or water-stressed +*Xf* plants.

The canes of both +*Xf* and –*Xf* water-stressed plants matured faster, becoming stiffer and more woody than those of the well-watered plants, based on the extent of periderm development up the canes. Stems of water-stressed + *Xf* plants became woody before the well-watered plants. Interestingly, in +*Xf* plants only the well-watered vines developed green islands, having an average of 2.1 ± 0.31 green islands per plant.

Vessel Blockage in Relation to Leaf Scorch Symptoms

Leaf scorch symptoms, in particular, have been considered a direct result of water deficits within the leaf, specifically due to clogged vessels limiting water transport. If leaf scorch is simply a matter of reduced water availability to the leaf margins, then we should be able induce leaf scorch symptoms by selectively severing veins to simulate xylem vessel blockage. To this end, experiments were conducted in which all veins but one were severed such that a single secondary leaf vein connected the two halves of a lamina and was the sole water source for the nearly-severed portion of the leaf. Nearly-severed leaf halves of vines experiencing low transpirational demand in the laboratory appeared turgid and showed no signs of necrosis for up to 36 days. In the greenhouse, under medium to high transpirational conditions, sections of leaves which received water via a single vein remained green and turgid (Fig. 3) for at least 30 days after the veins were severed**.** This was true for leaves of +*Xf* and –*Xf* grapevines alike. Significantly, leaf scorch symptoms of PD did not develop on any of the –*Xf* nearly-severed leaves. Even when these leaf sections did eventually dehydrate after approximately two months, the symptoms were similar to water deficit, not PD.

Figure 3. Turgid leaf of non-infected Chardonnay under moderate to high transpiration 13 days after all but one vein was severed, resulting in a single leaf vein connecting and supplying water to half of the leaf. Black arrow shows secondary vein supplying water to the nearly-severed leaf half.

CONCLUSIONS

In summary, water deficit clearly had an exacerbating effect on the symptom development of PD. Water-stressed +*Xf* vines displayed more extensive PD symptoms throughout the plant than did well-watered vines. Matchstick and leaf scorch symptoms moved up the canes more rapidly than in well-watered vines implying that the bacteria spread more rapidly throughout the plant under water deficit conditions, assuming bacterial proximity is necessary for symptom development. Importantly, with the exception of green islands, extended water deficit did not affect the nature of the PD symptoms. Indeed, in water-stressed +*Xf* plants, PD masked all of the symptoms of water deficit, except green islands, which occurred only in well-watered +*Xf* vines.

Detailed comparisons of the visual symptoms of PD and water deficit revealed that conclusions reached from earlier work, stating that water deficit causes PD symptoms, were not completely correct. The visual characteristics of +*Xf* vines were unique to PD and distinctly different from –*Xf* vines experiencing extended water deficit. The fracture zone at the petiole/lamina junction, common to all treatments, appears to be a plant response to stress and not specifically induced by bacterial infection. In contrast, the lack of an abscission zone in +*Xf* plants implies that the bacteria were in some way suppressing development of an abscission zone. Conversely, water deficit overcame the influence of *Xf* to prevent the occurrence of green islands, possibly by hastening periderm development. Considering that only well-watered +*Xf* vines developed green islands, water deficit could have masked the green island symptom of PD by inducing the periderm of +*Xf* water-stressed canes to develop faster than could the conditions necessary to impair periderm activity leading to green islands. This suggests that the bacteria are in some way inhibiting periderm activity at seemingly random locations.

Finally, based on the dramatic and sudden increase in the number of nonfunctional vessels which was caused by severing leaf veins, it seems clear that xylem vessel blockage, whether due to gums, tyloses or bacterial accumulation, is not responsible for leaf scorch symptoms and that *Xf* bacteria are able to affect plant responses in ways not involving altered vine water status. While occluded xylem vessels may worsen leaf scorch symptoms, several other factors, or combination of factors, may contribute. Ultimately, however, comparison of the leaf scorch symptoms of PD and the chlorosis of extended water-stressed leaves shows that *Xf* bacteria are able to produce, alter or eliminate signals that result in leaf scorch symptoms and that these signals can, to some degree, override signals controlling plant responses to water deficit. (A manuscript containing the completed study will be submitted to a peer-reviewed journal shortly.)

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FUNDING AGENCIES

Funding for this project was provided by the CDFA Pierce's Disease and Glassy-winged Sharpshooter Board.

EPIDEMIOLOGICAL ANALYSES OF GLASSY-WINGED SHARPSHOOTER AND PIERCE'S DISEASE DATA

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Reporting period: The results reported here are from work conducted from July 1, 2004 to October 8, 2004.

ASBTRACT
The progression of PD in vineyards and across a landscape is dependent upon factors related specifically to four components: GWSS, *Xylella fastidiosa* causing PD, grapes, and the environment. When conditions in all four of these areas are optimal, disease spreads with devastating consequence as in Temecula in the late 1990s. Conversely, sub-optimization within any of the four categories can slow or stop disease progress. The aggressive insecticide campaigns against GWSS are prime examples of creating this sub-optimal condition for disease spread. This single approach has been effective, but it may not be sustainable in reduced budget times. The science of epidemiology seeks to determine how the 4 components listed above interact, with the goal of creating long-term, sub-optimal conditions for disease spread. Achieving this goal will enable California producers to continue growing grapes in areas known to have PD and GWSS.

INTRODUCTION
Earlier studies pointed out the importance of the distribution of disease (Weltzien 1972, 1978) and insects (Southwood 1978), but mapping the distribution of disease and insect populations has not been applied to entomological and epidemiological studies until recently. This is mainly because there was a lack of suitable technologies or methods to map the distribution of insects and diseases in the field. Recently, the global positioning system (GPS), the geographic information system (GIS), and geostatistics have been applied to entomological and epidemiological. These technologies combined with advanced statistical methods can facilitate the making of distribution maps and the analyzing and modeling of the spatial phenomena represented on the maps.

OBJECTIVES
The overall goal of this research is to analyze the GWSS and PD data to investigate the relationship between GWSS and PD. The objectives of this research include,

1. Determine the spatial patterns and structures of GWSS and PD distributions, and use these analyses to create statistical distribution maps.
2. Analyze map correlations between GWSS abundance and incidence of PD.
3. Relate the epidemiology of GWSS-transmitted PD to environmental components, and identify characteristics of areas with rapid and slow PD infection rates.

RESULTS AND CONCLUSIONS
This project has just begun, so our report is preliminary at the present time. Prior to analyses, the GWSS and PD data need to be centralized into a geo-referenced database. Fortunately, there has been a tremendous and successful effort to maintain a weekly trapping effort for GWSS in areas of Kern, Tulare, and Ventura Counties. The data have been managed in a geographic information system (GIS) maintained by Rosie Yacoub of CDFA in Sacramento. We are working closely with Rosie to obtain trapping data from Kern County. Secondly, for certain areas there are crop layers that have been entered into the GIS, and we will work closely with the Kern County GIS group to obtain these layers. Within these two data sets we find

information related to two of the four epidemiological components (i.e., GWSS abundance and the agricultural environment). Data from the other two components (i.e., PD and grapes) also have been collected, largely by Barry Hill and Jennifer Hashim (Hill and Hashim 2002, Hashim and Hill 2003). These scientists have directed crews to survey hundreds of vineyards in Kern and Tulare counties over the past four years. Much of the data has been entered and managed in a GIS format at UC Berkeley under the direction of Maggi Kelly. We have begun the process of bringing the PD data together with the GWSS data and crop layers. Once the map databases are constructed and standardized, we will pursue the analyses phases of this project.

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FUNDING AGENCIES

Funding for this project was provided by the CDFA Pierce's Disease and Glassy-winged Sharpshooter Board.

AREA-WIDE EPIDEMIOLOGY OF PIERCE'S DISEASE IN THE COACHELLA VALLEY

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REPORTING PERIOD: The results reported here are from work conducted from May 1, 2001 to September 30, 2004.

ABSTRACT

This is a continuation of the epidemiology project that was initiated in 2001 in the Coachella Valley. Surveys in 2001 did not detect any Pierce's disease (PD). In 2002, we identified 2 infected vines in one vineyard and 1 infected vine in an adjacent vineyard. These were the first finds of PD in the area since 1983. Intensive surveys in these vineyards over the past 3 years have revealed a total of 16 infected vines. In June 2003, we found PD-infected vines in 2 additional vineyards. Further work in these vineyards has identified a total of 62 vines infected with PD. This past summer (2004), we again surveyed all vineyards in the Valley, finding PD-infected vines at 3 additional sites. Additional searches have identified a total of 19 infected vines in these three vineyards. With the finds this past summer, we now have identified 97 PD-infected vines from 7 vineyards. Except for the two infected vineyards identified in 2002, sharpshooter densities have been low near the sites that have PD.

Since the inception of this project in May 2001, we have used yellow sticky traps to monitor the spatial and temporal abundance of adult glassy-winged sharpshooters (GWSS), *Homoladisca coagulata* (Say) and native smoke tree sharpshooters (STSS), *Homoladisca liturata* Ball in the Valley. In 2001-2003, two peaks were identified in abundance; a broad-peak around a maximum abundance in July and a second smaller peak in winter. Summer densities in 2002 were higher than the same time in 2001 and winter counts in 2003 were higher than winter densities in 2002. This apparent increase in GWSS abundance was altered by the CDFA-sponsored vector control program being implemented through the Riverside County Agricultural Commissioner's Office. This program was initiated in the winter of 2003, and since then, very few GWSS adults have been caught on our traps. Relative densities of the STSS have remained constant throughout the 4-year study period.

INTRODUCTION

The Coachella Valley is home to 11,345 acres of table grapes; in 2003 harvested grapes from this region were valued at $115,939,900 (Riverside County Agricultural Commissioner, 2003). Pierce's disease first was identified in the Valley in 1983 (Goheen 1984), and from that time until recently, it has not been a concern to growers. When the GWSS was identified from the Valley in the early 1990s (Blua et al. 1999), growers became concerned, since this insect had been shown to be instrumental in the devastating spread of PD in the Temecula Valley in the late 1990s. At the request of the table grape growers, we initiated a study in 2001 to determine the spatial and temporal distribution of GWSS, and to identify the distribution of PD in the Valley. From that point in time to the present, we have continued our monitoring efforts, with the intention of describing the epidemiology of GWSS-transmitted PD in this area.

OBJECTIVES

The goal of our studies in the Coachella Valley is to describe the epidemiology of PD in the presence of GWSS, and to use this information to design management strategies to reduce disease spread.
Three objectives are pertinent to this report:

1. Determine the incidence and distribution of PD in the Coachella Valley.
2. Determine the spatial and temporal abundance of sharpshooters in the Coachella Valley.
3. Describe the epidemiology of PD in the Coachella Valley.

RESULTS AND CONCLUSIONS

Determine the incidence and distribution of PD in the Coachella Valley

For the past 4 years, we have searched for PD in the Coachella Valley. In 2001, we visually inspected 300 plants in each of 25 vineyards and all vines in a 60-acre vineyard proximal to an area that had PD in 1983. We collected 233 symptomatic samples and analyzed them with ELISA. None of these plants were positive for *Xylella fastidiosa*, the causal agent of PD. In 2002, we visually sampled 300 plants in each of 25 vineyards, and visually inspected 35,000 vines distributed throughout the Valley. We analyzed (by ELISA) 268 plants from these surveys, and found 2 infected vines in one field and 1 infected vine in an adjacent field. We analyzed (by ELISA) 268 plants from these surveys, and found 2 infected vines in one field and

1 infected vine in an adjacent field. Both fields were in the southeast corner of the Valley (Figure 1). The PD-strain of *X. fastidiosa* was confirmed in these plants with selective-media plating and PCR. These were the first post-GWSS PD finds in the Valley. Intensive sampling in these 2 fields over the past 2 years has found 13 additional vines infected with *X. fastidiosa*. In 2003, we visually inspected an estimated 616,400 vines and samples from 478 vines with suspected PD were subjected to ELISA. Five of these 478 vines were positive for PD. Four of these vines were at one field site and the 5th vine was at another site. Interestingly, neither vineyard was near the infected vineyards identified in 2002, and the fields were not near each other (Figure 1). One of the vineyards was in a fairly isolated location on the west side of the Valley. Further searches of the two infested vineyards found no additional PD infection at one of the sites, however work at the site on the west side of the valley has identified a total of 61 infected vines. We are in the process of characterizing this field to determine the spatial pattern of infection. In the 2004 survey, we observed an estimated 571,861 vines and collected 187 samples to assay for PD. From these assays we identified 5 infected vines, adding 3 vineyards to our list. These vineyards were located in the east-central part of the valley with an additional find in the far southwest corner of the Valley (Figure 1). Further research has identified a total of 19 infected vines from these three vineyards. We are in the process of determining the distribution of PD-infected vines in these vineyards.

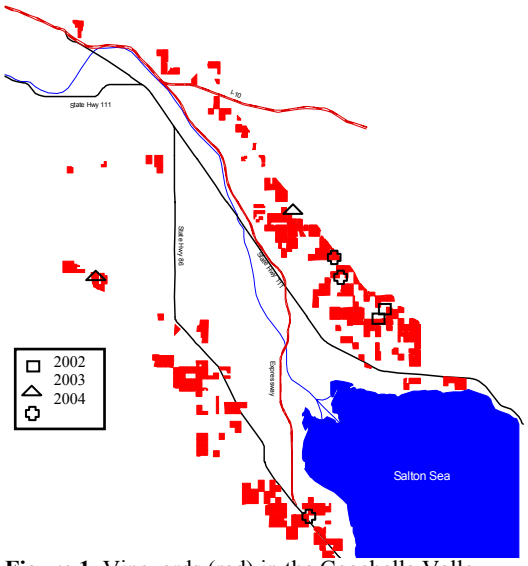

Figure 1. Vineyards (red) in the Coachella Valley, and sites where PD was confirmed in 2002, 2003, and 2004.

Spatial and temporal abundance of sharpshooters

Yellow sticky cards have been used to trap GWSS and STSS adults from May 2001 to the present. These 156 traps are distributed uniformly at one-mile intervals throughout the Coachella Valley. Traps are checked weekly and the total numbers of sharpshooters are recorded.

We discuss the trap data in two distinct time periods. The first, from May 2001 through January 2003, preceded the CDFA treatment program in citrus while the second period from February 2003 to the present has been during the implementation of this areawide program. During the early part of this period, GWSS vastly outnumbered STSS (Figure 2A). While average densities did not exceed 3 GWSS per week, some sites had very high GWSS catches; up to 160 insects per week were trapped (Figure 2B). During the second period of trapping, STSS numbers remained consistent with previous years, and even increased in 2003 (Figure 2B). A few sites reached high densities of STSS, nearly as abundant as the GWSS peaks in 2002. Presently, STSS outnumber GWSS in the Valley. The reason for these seasonal dynamics is that the CDFA treatment program specifically targets citrus, a preferred host of GWSS during certain times of the year. STSS, on the other hand, utilizes a number of desert scrubs and riparian plants, thus its densities have been largely unaffected by the treatment program. STSS is a known vector of PD, but it is not clear how important it is in the epidemiology of the disease.

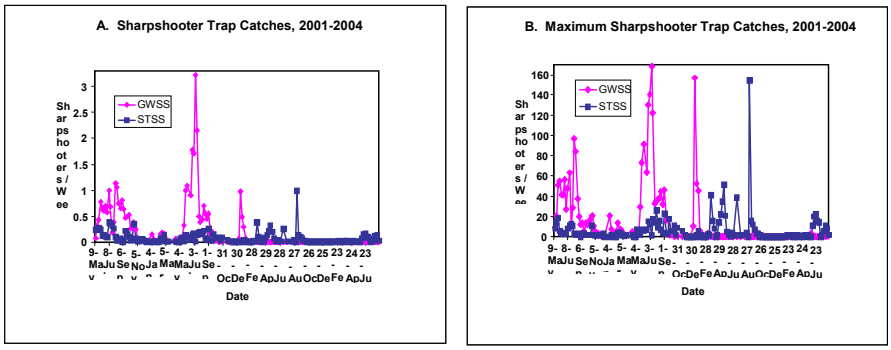

Figure 2. (A) Average number of GWSS (pink) and STSS (blue) trapped per week from 2001 - 2004 in the Coachella Valley. (B) Maximum number of GWSS (pink) and STSS (blue) trapped per week.

GWSS Seasonal Abundance

From 2001-2003, two peaks of adult activity were identified; a broad-peak centered around a maximum abundance in July and a second smaller period of activity in January and February (Figure 3). Summer densities in 2002 were higher than the same time in 2001 and winter counts in 2003 were higher than winter densities in 2002. This apparent general increase in

GWSS abundance was altered by the CDFA-sponsored vector control program being implemented through the Riverside County Agricultural Commissioner's Office. Treatments from this program were initiated in the winter of 2003, and since then, very few GWSS adults have been caught on our traps (Figure 3).

STSS Seasonal Abundance

Generally, trap counts of STSS peaked at about 1/3 the densities of GWSS in 2001 and 2002 (Figure 3). However, in 2003, average densities equaled GWSS, and at certain sites, there were far more STSS than GWSS (Figure 2B). Since STSS have non-citrus hosts throughout the Valley, they have not been affected by the treatments in citrus. It is unclear at this time what role this species may play in the epidemiology of PD in the Coachella Valley, but we will be investigating this as we continue data analysis

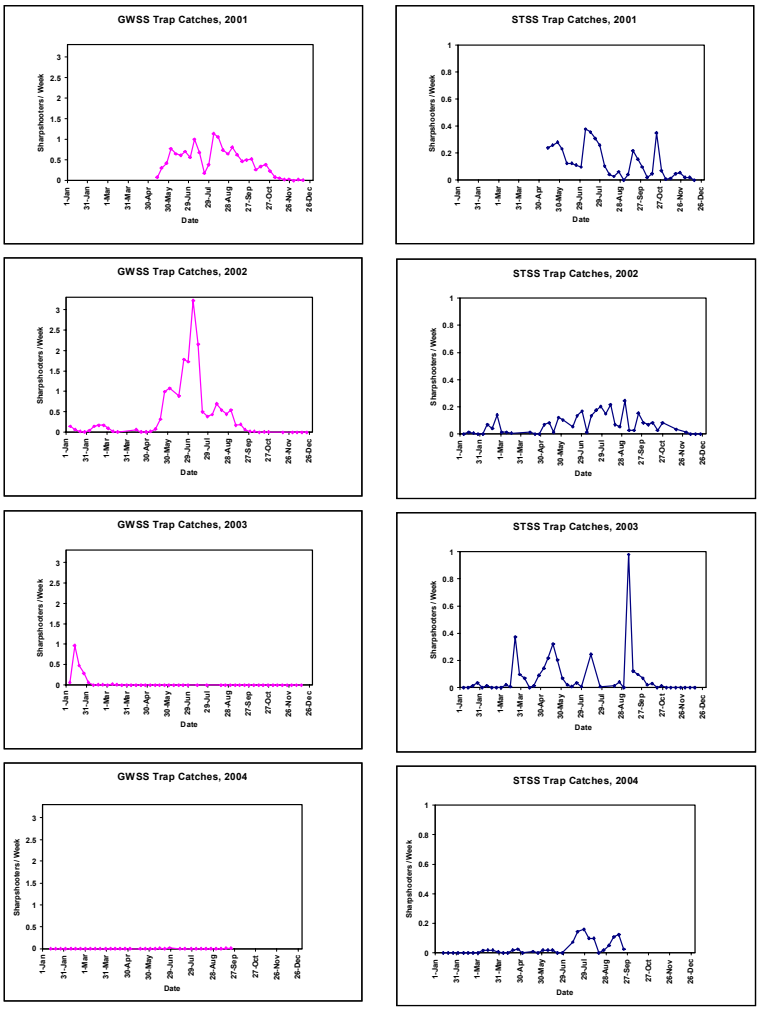

Figure 3. Average number of GWSS (pink) and STSS (blue) trapped per week from 2001 – 2004 in the Coachella Valley displayed for each year.

Describe the epidemiology of PD in the Coachella Valley

Since we have so few sites infected with PD, and the number of infected vines at each site is low, it is difficult to draw conclusions about the epidemiology of PD in this area. However, we calculated the maximum numbers of GWSS and STSS adults caught on yellow traps within one mile of the 7 fields in which we have found PD, to determine if any relationships were apparent. From this exercise, we present several preliminary observations. First, we observe the highest incidence of PD was not in an area where we caught large numbers of GWSS (Figure 4) or STSS (Figure 5). In fact, the heaviest PD vineyard, found in the northwest part of the Valley, has had maximum numbers of GWSS and STSS of 1 per week since we started trapping in 2001. In this field, we suspect other sharpshooter species are involved with PD spread, or our trapping program is too coarse to detect GWSS and STSS. Second, the two vineyards in which we identified PD in 2002 were in areas that were heavily infested with GWSS (Figure 4). If the trend of increasing GWSS from 2001 to 2002 (see Figure 3) had been allowed to continue in 2003 (in the absence of the CDFA spray program) one might have predicted spread of PD from these fields to neighboring vineyards. Because this did not materialize, the evidence suggests that the areawide program effectively impeded PD spread in this area of the Coachella Valley. Finally, while the number of fields in which we have found PD remains low, relative to other areas of the state, each year we have found additional vines with PD. Having learned from the epidemic that occurred in Temecula, we suggest continuing the sharpshooter and PD monitoring efforts to insure that this scenario is not repeated in the Coachella Valley.

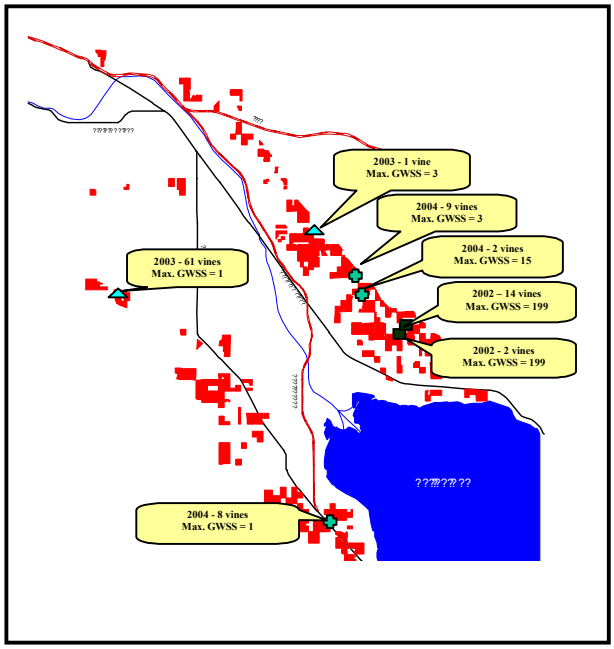

Figure 4. Sites with PD and maximum GWSS numbers in the Coachella Valley from 2001-2004.

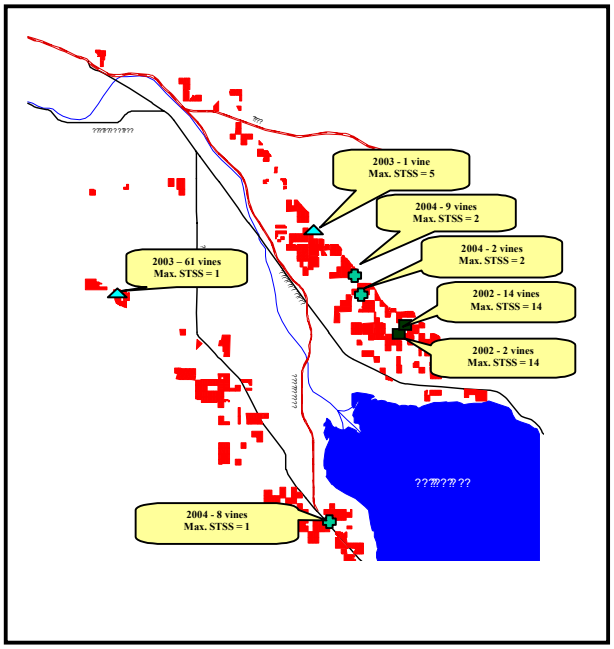

Figure 5. Vineyards (red) in the Coachella Valley, and sites where PD was confirmed in 2002, 2003, and 2004.

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FUNDING AGENCIES

Funding for this project was provided by the California Desert Grape Administrative Committee, the University of California Pierce's Disease Grant Program, and the California Department of Food and Agriculture.

IMPROVING OUR UNDERSTANDING OF SUBSTANCE TRANSPORT ACROSS GRAFT UNIONS

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ABSTRACT
Researchers seeking to genetically-engineer grapevine rootstocks in order to affect Pierce's disease (PD) resistance in scion cultivars know very little about the transport of substances produced by foreign genes across the graft union. Our project seeks to understand how protein size and concentration may affect protein transport from a rootstock to a scion. We possess genetically engineered lines of Chardonnay, Merlot and Chancellor that produced proteins ranging in size from 29 to 97 kDa. These proteins can be readily detected by established techniques. Lines will be identified with low and high protein production potential in their root tissues, and graft combinations will be created with non-transgenic Chardonnay scions. Xylem sap will be collected from the scion and tested for the presence of the transgenic proteins. Given that Xylella fastidiosa causing plugging of xylem tissues, the results of xylem sap testing will be directly applicable to efforts to develop PD resistance inducing rootstocks.

INTRODUCTION
One approach being utilized to develop a long-term solution to Pierce's disease is the development of transgenic PD resistant versions of important wine and table grape varieties. The development of each transgenic cultivar will require a concentrated effort and significant amounts of technical expertise, testing, and funding. To bring each successful product to market, and to pass regulatory agency approval for transgenic crops, also will require a great deal of time and funding. This would be required for each of dozens of scion varieties.

A rootstock-based approach provides a potentially excellent alternative. In theory, a transgenic rootstock would confer PD resistance to its non-transgenic scion. Advantages include: 1) many fewer rootstocks will need to be transformed as compared to the dozens of table grape and wine grape varieties that would need to be altered, 2) consumers might be more accepting of wines produced from non-transgenic scions even if they are grafted on transgenic stocks; and 3) in general, it has been technically easier to transform rootstocks than scion varieties. Before this approach is successful, however, our understanding of the biology of the graft union and the types of substances that can be successfully transported from rootstocks to scions must be improved.

Water, mineral nutrients, hormones, carbohydrates, and other compounds are all known to move, via both xylem and phloem, from rootstocks across graft unions into scions of woody plants. To date, however, there is little evidence available to show whether a transgenic protein can move from the rootstock into the scion in a grafted woody plant. In recent work with grapevines, Meredith and Dandekar (2003) showed that pear polygalacturonase inhibiting protein (PGIP), with a size of 36.5 kDa, could be detected in xylem sap of non-transgenic scions grafted on transgenic stocks engineered to produce this protein. Of great relevance to this proposal, we noted that protein movement into the xylem occurred even without a specific signal targeting it to the extracellular spaces or to the xylem. Imidacloprid (a small compound with molecular weight of approximately 0.25 kDa) and other systemic insecticides applied to the soil are taken up by the roots of grapevines and move from root systems into the scion (Toscano et al. 2003). The present project will investigate aspects of plant physiology critical to determining the potential for deploying transgenic rootstocks for PD management.

It is possible that the size of a transgenic protein produced in a rootstock influences its transport to the scion. For example, large proteins might be less likely to be transported than small proteins. Understanding the relationship between size and movement will allow us to more efficiently test anti-PD compounds. If transgenic proteins are transported across the graft union, their concentration in the roots might be higher than their concentration in the scion. Since there is likely to be a threshold concentration for PD control provided by a given compound, it will be critical to understand the relationship between concentration in the rootstock and concentration in the scion.

By studying non-transgenic scions grafted on transgenic rootstocks in the course of this project, we expect to learn whether the transgenic proteins can move from the rootstock to the scion, whether molecule size affects transport, and whether substance concentration in the rootstock affects levels found in the scion.

OBJECTIVE
Determine the relationship between protein molecule size and concentration in grapevine roots and its ability to move from a grapevine rootstock to a scion across a graft union.

RESULTS
This project is just getting underway, thus, rather than present non-existent research results, an outline of our research plan is presented here.

The following transgenic grapevines are available for use:
1. Two lines of Chancellor transformed with an NPT-II/GUS gene fusion producing a fused protein product. One line strongly expresses the *gus* reporter gene (*uidA*) in all tissues, while the other line shows no GUS expression, even though the gene is present.
2. Multiple lines of Chardonnay and Merlot producing both NPT-II and endochitinase.
3. A series of lines of Chardonnay producing NPT-II along with one of three antimicrobial peptides (AMPs).

All of these lines produce transgenic products under control of constitutive promoters. In cases 1 and 2 above, the CaMV 35S promoter was employed, whereas in case 3, NPT-II was downstream of an *Arabidopsis* ubiquitin promoter. The CaMV 35S promoter was used by Meredith and Dandekar (2003), who showed that PGIP protein from rootstocks could be detected in xylem sap. The NPT-II/GUS gene fusion product in Chancellor was shown to express in root tissues (Striem et al. 2000), but will require re-testing to make sure that protein production has not been lost since these tests were run. We will need to test the other lines (2 and 3 above) to determine the transgenic protein concentration in their roots. The size of the transgenic product molecules varies: NPT-II is ~280 amino acids (aa) (29 kDa); endochitinase is 424 aa (42 kDa); the NPT-II/GUS bifunctional fusion protein has 885 aa (97 kDa).

We will examine root tissues from separate lines of each of the three types of transformed vines listed to determine gene transcription and transgenic protein concentration via established procedures. To test for gene transcription we will use semi-quantitative RT-PCR (Vidal et al. 2003). Transgenic protein concentrations will be determined using standard methods already in use in our lab. We will identify lines with high and low concentrations of transgenic proteins for further use in this project.

The transgenic lines with high and low concentrations of transgenic proteins, along with negative controls, will be bench grafted as rootstocks to non-transgenic Chardonnay scions. The grafted vines will be grown in a greenhouse. Once the grafted vines have been established and their shoots have grown to 50 cm, the non-transgenic Chardonnay scions will be examined for presence of transgenic proteins. Leaf tissue as well as xylem sap will be tested. Samples will be collected under sunny, warm conditions conducive to transpirational pull through the xylem.

Outline of rootstock/scion combination planned:
13 rootstock/scion combinations planned, including control
10 vines of each combination x 13 combinations = 130 vines total planned
Control rootstock: Non-transgenic Chardonnay (to be grafted to non-transgenic Chardonnay)

Experimental rootstocks:
(Each rootstock will be grafted to non-transgenic Chardonnay scions.)
Chancellor, high NPT-II/GUS fused protein product concentration in roots (35S promoter)
Chancellor, transformed vine with no GUS expression in roots (35S promoter)

Chardonnay, high NPT-II concentration in roots (Nos promoter)
Chardonnay, low NPT-II concentration in roots (Nos promoter)

Chardonnay, high NPT-II (*Arabidopsis* ubiquitin promoter)
Chardonnay, low NPT- II (*Arabidopsis* ubiquitin promoter)

Chardonnay, high endochitinase concentration in roots (35S promoter)
Chardonnay, low endochitinase concentration in roots (35S promoter)

Merlot, high NPT-II concentration in roots (Nos promoter)
Merlot, low NPT-II concentration in roots (Nos promoter)

Merlot, high endochitinase concentration in roots (35S promoter)
Merlot, low endochitinase concentration in roots (35S promoter)

Additional controls will include own-rooted transgenic vines to be used to test for presence of foreign protein in the xylem sap.

CONCLUSION

The success of this project will rest on the careful, methodical characterization of foreign gene products. This project will not involve the speculative and lengthy creation of novel transgenic grapevines, but rather uses pre-existing transgenic grapevines in order to investigate the potential for transgenic rootstocks to deliver proteins to their non-transgenic scions.

Based on the evidence from the movement of imidacloprid and PGIP in grafted grapevines, it is likely that transgenic grapevine rootstocks will transmit transgenic proteins to their non-transgenic scions. However, it is premature to speculate concerning the time frame for reduction to practice in the form of a novel PD management strategy. We emphasize that this study is intended to investigate the biological principles of protein transport via xylem in grapevines, a topic that has been studied very little in the past. By understanding the potential of a transgenic grapevine rootstock to move proteins into a non-transgenic scion, scientists will be better equipped to investigate and develop novel PD management strategies.

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FUNDING AGENCIES

Funding for this project was provided by the University of California Pierce's Disease Grant Program.

MECHANISMS OF PIERCE'S DISEASE TRANSMISSION IN GRAPEVINES: THE XYLEM PATHWAYS AND MOVEMENT OF *XYLELLA FASTIDIOSA*. PROGRESS REPORT NUMBER TWO: GREEN ISLANDS AND MATCHSTICKS

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Reporting Period: The results reported here are from work conducted from October 2003 to September 2004.

ABSTRACT
During this period our focus was the comparative xylem anatomy of a resistant species, *Muscadinia rotundifolia* cv Cowart and a susceptible species, *Vitis vinifera* cv Chardonnay. When infected by *Xylella fastidosa* both species produced tyloses (parenchyma ingrowths into tracheary elements) and gums; *M. rotundifolia* tended to have fewer tyloses. The resistant species also had narrower vessels, but otherwise xylem anatomy was similar to *V. vinifera*. Fluorescently tagged beads were loaded into both species. Beads traveled through the stem xylem in both, but did not move into petioles in these experiments. Tyloses were first apparent 24 hours after pruning in both species and most vessels were blocked in both after eight days of pruning. This suggests that the mechanism to form tyloses in both species is similar, although the resistant species tended to show fewer tyloses in response to *Xf*. Two symptoms, green islands and matchsticks are reported in this study. Green islands formed as a result of incomplete initiation of the phellogen. In regions of the stem where a phellogen and subsequent periderm arose, immediately exterior tissue was cut off, causing it to brown. In regions of the stem where no periderm is formed, the exterior tissues remained green. Consequently, the stem is mottled with both green living epidermis and brown dying epidermis as determined by the presence or absence of an underlying periderm. Matchsticks formed when the leaf lamina separated from the petiole, and the petiole remained attached to the stem. Lamina broke off from the petioles consistently in a fracture zone where xylem from the petiole anastomoses into the five major veins of the leaf. No separation layer was found to explain this pseudoabscission.

INTRODUCTION
Xylella inoculation of stem xylem precedes a relatively rapid movement of bacteria through the hydraulic network (system of xylem) to the leaves. Once bacteria moving in the transpiration stream enter regions of the hydraulic network that contain narrow tracheary elements and terminal tracheary elements (i.e. shorter vessels in petioles and leaves), bacteria may be 'filtered out', accumulate, and become embedded in a gel which effectively blocks water flow in that conduit. Tyloses are cell wall extensions of xylem parenchyma cells into tracheary elements. Tylose formation in the stem coincides with bacterial infection, but at least initially, is not present to such a degree that bacterial movement is apparently prevented or that the water supply to distal tissues is restricted to levels causing visual symptoms. Additionally, bacteria can move relatively quickly from an inoculated shoot to another shoot via the subtending trunk.

A similar understanding of the progression of events is needed for resistant varieties and species in order to localize investigations into the mechanism(s) of resistance. The anatomical symptoms of PD, xylem occlusions of gums and tyloses, are well documented in both susceptible (Esau 1948) and resistant plants (Mollenhauer and Hopkins 1976). However, it is not clear whether these occlusions are related to susceptibility or resistance. Only the susceptible plants express leaf scorch and eventual death, and these disease symptoms are widely understood to be water stress (Hopkins, 1989). Sufficient occlusions would produce water deficits downstream. Plants resistant to PD may remain healthy despite systemic populations of *Xylella* present in the vascular tissue because tylose and gum formation are not induced compared to susceptible varieties. Alternatively, the occlusions may prevent the movement of the bacteria, and comparative studies report that the frequency of occlusions is greater in resistant than in susceptible varieties (Fry and Milholland, 1990). Thus, resistant varieties or species may restrict *Xf* to regions of the hydraulic network proximal to the point of inoculation, either by occlusions or other mechanisms described below. In the reported experiments, we have initiated those studies. Regardless of whether resistance is dependent upon controlling the movement of *Xf*, Pierce's Disease is fatal because *Xf* becomes systemic. Host species in which *Xf* is confined to specific tissues, or is otherwise prevented from becoming systemic, do not display symptoms of PD (Hill and Purcell, 1995).

It is generally accepted that the fatal nature of Pierce's Disease is a result of the bacteria becoming systemic and water stress becoming increasingly severe until the plant is no longer able to function (Goodwin et al., 1988). However, the classic PD symptoms: patchy leaf chlorosis, persistent "green islands" on stems, and "matchsticks" (leaf abscission at the petiole/blade junction) are not generally observed in vines exposed to water stress alone. If the symptoms of PD are not, in fact, a result of water deficit, then studies relying on the assumption that water stress is the ultimate killer of plants suffering from PD, may result in misleading information and add years to finding solutions to the PD problem. Our second annual report addresses these concerns.

OBJECTIVES

1. Study the progression of anatomical symptoms created by *Xf* over a time-course in a PD resistant grapevine species, *Muscadinia rotundifolia* cv Cowart.
2. Determine the hydraulic architecture of a PD resistant species, *M. rotundifolia*.
3. Study the integrity of pit membranes of both PD susceptible *Vitis vinifera* cv Chardonnay and resistant *M. rotundifolia* by following the in situ movement of fluorescently tagged beads.
4. Determine the rate of tylose development from wounding in both PD *V. vinifera* and *M. rotundifolia*.
5. Study the developmental anatomy of green island and matchsticks in *V. vinifera*.

RESULTS

1. PROGRESSION OF PD SYMPTOMS IN RESISTANT SPECIES

The progression of anatomical symptoms created by infection by *Xf* was studied along a time-course as was previously conducted with *V. vinifera* (Stevenson, Matthews and Rost, 2004). Similar experiments were conducted with PD resistant *M. rotundifolia* in an attempt to discern quantitative or qualitative anatomical differences in a six-month post-inoculation period. The development of symptoms in the resistant species was qualitatively similar to that in resistant species (development of tyloses in stems, development of gums in petioles), however the rate of development and overall occlusion created by these symptoms was dramatically lower. In the resistant species overall occlusion was minimal (<5% of vessels) after nearly four months (Figure 1), whereas in susceptible species overall occlusion was great (~50% of vessels).

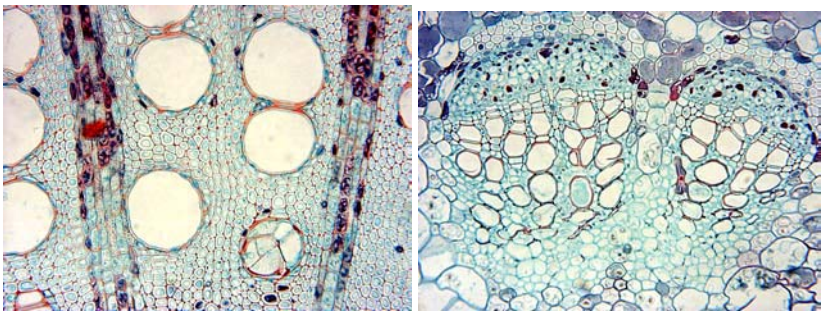

Figure 1. Minimal vessel occlusion in stem (left) and petiole (right) xylem of *M. rotundifolia* 122 days post-inoculation with *Xf*.

2. HYDRAULIC ARCHITECTURE OF RESISTANT SPECIES

The general hydraulic architecture of PD susceptible *V. vinifera* has been presented (Stevenson et al. 2004). Similar studies were conducted with PD resistant *M. rotundifolia* in an attempt to elucidate anatomical differences that may explain PD susceptibility or resistance. Regions of grapevine stem were serially sections to follow xylem arrangement in the node and internode. No significant differences were observed in the organization of stem xylem or in the divergence of xylem to lateral organs between resistant and susceptible species. The only difference found between the species was that *M. rotundifolia* possessed significantly narrower vessels than were found in *V. vinifera*. The difference may be contribute to restricting bacterial movement. Narrow vessels may cause bacterial conglomeration closer to the point of inoculation and prevent long distance bacterial seeding. Additionally, narrower vessels have less overall pit surface, which may further reduce the number of alternative pathways available to bacteria. Both of these proposals require further investigation.

3. PIT PROPERTIES OF SUSCEPTIBLE AND RESISTANT SPECIES

Preliminary investigations were conducted towards the study of the characteristics and integrity of pit membranes in susceptible and resistant grapevine species. The movement of *Xf* bacteria in the host is potentially facilitated by damaged pit membranes of grapevine, compromised either in development, or as a result of frequent cavitation/refilling cycles (Hacke et al. 2001, Sperry et al. 1987).

A. Movement of Fluorescent Beads

Fluorescent beads of similar size to *Xf* bacterial cells were injected into stem xylem of *V. vinifera* and *M. rotundifolia* (Figure 2). The distance of bead travel from the inoculation point was recorded as an indicator of vessel length and pit membrane integrity. Beads were observed to travel similar distances in both species (*V. vinifera* 1.6 ±0.5 nodes, *M. rotundifolia*1.8 ±0.4 nodes). The relatively short distance that these beads traveled indicates a general integrity within the vessel pits and is evidence against pit damage commonly occurring. Beads were never observed to pass into petiole xylem, which suggests

that some pit membrane disruption, is required for bacteria to colonize petiole and leaf tissue (Stevenson, Matthews and Rost, 2004a).

B. Resin-casting and Macerations

Resin casts were made of the internal spaces of vessel lumina and pit surface morphology in both *V. vinifera* and *M. rotundifolia* (Figure 2). Superficially, no differences were seen in pit patterns, pit integrity, or relative pit surface area between the species. Further study is required to investigate subtle characters of pit membranes (ex. total pit membrane area, dimensions of pit apertures) that may facilitate pit membrane disruption by bacteria.

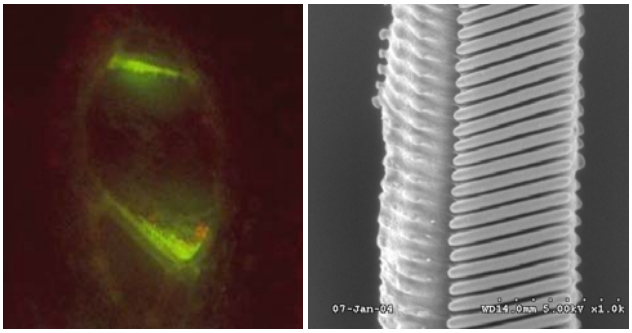

Figure 2. Fluorescent beads within stem xylem of *V. vinifera* used to mimic movement of passive bacterial cells (left), and resin casts of xylem vessels from *M. rotundifolia* to show fine detail of pit surfaces (right).

4. TYLOSE DEVELOPMENT

A. Rate of Tylose Development

A working hypothesis was developed that differential susceptibility to PD among grapevine species may involve differences in the rate of tylose development. The rate of tylose development was studied in both resistant and susceptible grapevines following wounding (pruning) injury. Tylose development was then observed allowing one, four, and eight days for tyloses to develop. Initial tylose development was found within a day, about half of the vessels were occluded by day four, and at day eight, most vessels of the stems were observed to be significantly blocked by tyloses (Figure 3). No superficial difference was seen between the rate of tylosis in PD susceptible *V. vinifera* and resistant *M. rotundifolia* at any of the time intervals, however, further quantitative analysis is necessary.

B. Vitality of Tyloses and Paratracheal Parenchyma

The presence of living cells surrounding the vessels during tylose formation following pruning was studied using the vital stain fluoroscein diacetate. This technique was used to discern a correlation between the amount of tylose occlusion found in the vessel and the number of vital paratracheal cells surrounding that vessel, and whether the number of vital paratracheal cells was significantly greater in PD susceptible grapevine species. Both resistant and susceptible grapevines were observed in this manner over the eight-day time course described in 4A. No superficial differences were seen in the vitality of paratracheal parenchyma surrounding vessels in the two species, however greater quantitative analysis is required. Overall, tyloses fluoresced greatly, indicating vital development, whereas paratracheal cells fluoresced only occasionally (Figure 3). These results suggest that very few active paratracheal cells are required to result in significant tylose development.

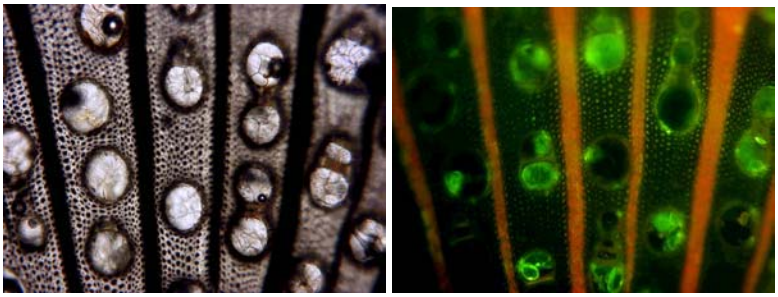

Figure 3. Micrographs of similar grapevine stems eight days following pruning. A bright field light micrograph (left) shows significant occlusion by tyloses at this interval. A fluorescence micrograph (right) shows fluorescent green vital staining predominantly by tyloses, but occasionally by paratracheal

5. DEVELOPMENTAL ANATOMY OF MATCHSTICKS AND GREEN ISLANDS

The development of the external visual PD symptoms of matchsticks and green islands was studied from a anatomical perspective (Stevenson, Matthews and Rost 2004b).

A. Matchsticks

Matchsticks result from pseudoabscission of the leaf lamina from the petiole. Following significant leaf scorching, the lamina breaks from the petiole at a predictable fracture zone. No separation zone develops as is common with typical leaf abscission, and hence this process is described as pseudo-abscission. Following pseudoabscission, exposed petiole tissues dehydrate and blacken to take on the appearance of a burnt matchstick. Occasionally, a wound periderm will form near the fracture zone following pseudoabscission. When this periderm forms, dehydration of the petiole is minimal. The process of matchsticking has never before been described anatomically.

B. Green Islands

Green islands arise from the incomplete development of the deep-seated phellogen (cork cambium) in *V. vinifera*. In regions of the stem where the phellogen arises and produces subsequent phellem (cork), external tissues (phloem, cortex, epidermis) are cut off from their nutrient sources and begin to die and brown. The juxtaposition of stem regions with active phellogen, and the juvenile character of no phellogen, creates green islands. It is unknown whether green regions are delayed in their development, or whether brown regions display advanced development. No obvious correlation was seen in the level of vessel occlusion proximal to green or brown regions. Additionally, periderm formation was observed in *M. rotundifolia*. Periderm formation in this species is subepidermal (vs. deep-seated) and consequently green islands may not form in this species (Stevenson et al. 200xC). This is important point for researchers using green islands as an indicator of PD resistance.

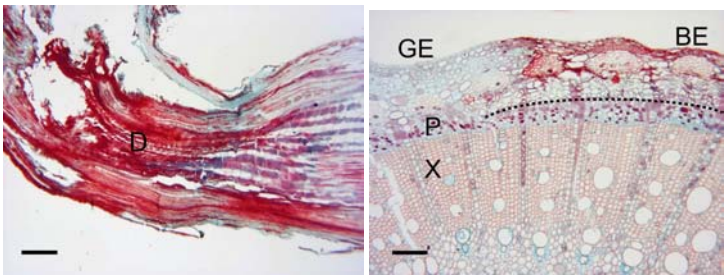

Figure 4. Longitudinal section through a matchsticked petiole (left) displaying basipetal dehydration (D) following pseudoabscission. Transverse section through a stem with green island (right) showing regions of green epidermis (GE) and brown epidermis (BE) created by presence of absence of phellogen initiation.

CONCLUSIONS

1. The development of tyloses and gums in response to *Xf* infection were qualitatively similar in the resistant *M. rotundifolia* cv Cowart and the susceptible *V. Vinifera* cv Chardonnay, although the resistant species tended to form fewer tyloses.
2. The only observable difference in hydraulic architecture was that the resistant species had narrower vessels.
3. Fluorescent beads were loaded into stems of both species. Beads moved approximately the same distance (~1.6-1.8 nodes) and in both cases did not enter into petioles.
4. Tyloses were first seen about 24 hours after pruning in both species. After four days about 50% of vessels were blocked. By eight days most vessels were blocked in both species.
5. Matchsticks formed in *V. vinifera* leaves after several days of *Xf* infection. This symptom consisted of the pseudoabscission of the petiole from the leaf blade. Green islands are green areas of the stem created by incomplete formation of periderm in infected plants.

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FUNDING AGENCIES

Funding for this project was provided by the CDFA Pierce's Disease and Glassy-winged Sharpshooter Board.

MAGNETIC RESONANCE IMAGING: A NONDESTRUCTIVE APPROACH FOR DETECTION OF XYLEM BLOCKAGES IN *XYLELLA FASTIDIOSA*-INFECTED GRAPEVINES

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INTRODUCTION

Results from Pierce's disease (PD) research programs led by Matthews, Rost and Labavitch (reported in 2001, 2002 and 2003 in San Diego) have provided substantial support for the idea that obstructions in the vine's water-transporting xylem tissue develop rapidly post-inoculation, before an appreciable bacterial population has been established. The results also strongly suggest that these obstructions, and likely other aspects of the PD "syndrome", result from the grapevine's active responses to the presence of *X. fastidiosa* (*Xf*), rather than to direct "action" by the bacterium. Thus, careful analysis of the timing of changes in xylem element anatomy and function relative to *Xf* introduction, as well as to external symptoms of disease development, is important for establishing reliable indicators of the "stage" of PD development. The analyses done thus far have been based on destructive tissue sampling. Such sampling can be particularly "blind" when it is done on vines in which (based on our earlier results) internal symptoms of PD are present but external, visible symptoms are not yet present.

In the report of the year 1 work of our study (Shackel and Labavitch, 2003), the success of Mr. Pérez and Dr. Walton in imaging non-functional vessels in the stems of PD-infected and ethylene-treated grapevine stems was demonstrated. In this report we elaborate on those studies, showing that locations of reduced vine water transport capacity, as determined by non-destructive MRI analysis, is correlated with the locations of PD and ethylene effects on vessel functionality (destructive analysis). In addition, because interpretation of the meaning of the MRIs with respect to the anatomy and functioning of vessels is a crucial aspect of our work, we have described the methodology used to validate our approach to obtaining the relevant information from the MRIs.

OBJECTIVES

1. Optimize the use of MRI (Magnetic Resonance Imaging) and to spatially visualize altered water movement in grapevines.
2. Test correlations of observed vascular system obstructions (based on grapevine dissection and microscopy techniques) with predictions based on MRI data.
3. Use MRI to follow the development of grapevine obstructions over time in vines infected with *X. fastidiosa* or treated with ethylene, bacterial wall-degrading enzymes or plant cell wall oligosaccharides, all of which may be important intermediates in regulating the vine's response to infection and the eventual development of PD symptoms.
4. Use NMR imaging to determine whether localized xylem cavitation occurs at the site and time of *X. fastidiosa* inoculation or introduction by the glassy-winged sharpshooter.

RESULTS

Optimization of the Use of MRI for Visualizing Water Transport Deficiencies in PD-Infected Grapevines.

Progress on this objective has been delayed because a supplier for a key electronic element of the new MRI probe that has been designed for use with grapevines no longer provided a key part. The parts are all now available and development of the new probe is underway. We are proceeding with the testing of aspects of the PD model using the NMR instrument in its more conventional configuration.

MRI Will Show Non-functional Sections in the Xylem of a PD-infectedGgrapevine Stem.

Usually the techniques to evaluate xylem function are destructive. Magnetic Resonance Imaging (MRI) allows us to visualize vessels that are functional and full of movable water. Functional vessels appear as bright spots in an MRI view of the stem cross-section; non-functional vessels lack water and appear as dark spots in the area of the stem where water-conducting cells are found. Figures 2a & 2b show the difference in the distributions of functional vessels in an infected vine at a point where leaf symptoms of PD are apparent (Figure 2a) and nearer to the stem apex at a point where the leaves show no sign of PD symptoms (Figure 2b). Compare these images with that for a healthy vine (Figure 3a). Cavitation of xylem vessels is also of

potential importance in PD development. Our analysis can reveal vessels that have cavitated. Figure 3 shows functional vessels in an intact stem, and empty vessels after the stem is severed to cause cavitation, and that cavitated vessels can be re-filled with water under pressure. When we have the optimized MRI probe we will develop a series of image sets taken along the lengths of vines at intervals following water (control) and *Xf* inoculation to give a time course of PD development. However, at this point we do not have images for a full time course.

MRI is capable of showing xylem disruption and non-functional vessels well before external symptoms appear in infected plants. Figures 4 and 5 show images for the length of control (buffer-innoculated) and infected (*X. fastidiosa*-innoculated) vines six months after inoculation. MRIs of the control-inoculated vine show defined xylem rays, in which individual vessels can be clearly observed. As in previous experiments, stem cross section MRIs of infected plants (Figure 5) show that major sectors of the xylem appear dark, indicating that they are no longer water-filled (Note: the magnetic signal is lost in cavitated vessels). Furthermore, MRIs of plants infected with *Xf* become less sharp, making it more difficult to discriminate structure, particularly of individual, probably still functional, vessels. Efforts to explain this will be a feature of the work as this project continues. MRI also has been used to follow changes in the functionality of the xylem of plants exposed to ethylene in enclosed chambers (10 ppm for 48 hours). We previously described the progressive development in time of "dark sectors" in the xylem of ethylene-gassed, presumably indicating vessels no longer involved in water transport. This new set of experiments has allowed us to confirm that, after 6 months of exposure to ethylene, gassed plants show progressive xylem disruption along the stem (Figure 6). Most of the damage is localized close to nodes/internodes that had just developed in the stem growth tip at the time of ethylene treatment and had then expanded in the intervening six months prior to our observations. The MRIs show "dark sectors" in those internodes. These sectors decrease are less extensive in internodes below and above the internodes that were in the growth tip at the time of treatment; that is, internodes formed after the time of treatment and already partially elongated, respectively when ethylene as applied. As in *Xf*-infected plants, MRIs of ethylene-treated plants are less sharp than images of control plants (Figure 6).

The impression of a loss in xylem function that is given by the MRIs of *Xf*-inoculated and ethylene-gassed vines can be correlated with a decrease in the hydraulic conductivity of internodes. This is tested by determining the rate of movement of pressurized water through stem segments (Figure 7). Similarly, stems of treated vines showed an increase in the hydraulic resistivity (the inverse of conductivity) relative to the controls (Figure 8), although this difference was statistically significant only for the ethylene experiment. The lack of statistical difference in the inoculation experiment is mainly due to the great variability found in the hydraulic resistivity of inoculated plants. In turn, this might be explained because these vines were in a gradation of early stages of PD infection when examined (they were not showing external symptoms). While there is some correlation between the MRIs showing localized areas of empty vessels and reduced hydraulic conductivity in regions of infected stems, the correlations are not perfect. This is due to at least two factors that will be tested more fully in our continuing work. First, an empty vessel shown in the MRI at one level in the plant's stem could be the result of a vessel obstruction or cavitation above or below the point on the stem where the MRI observation was made. There may be no actual impediment to water flow in the empty vessel at the level at which it is being imaged. Thus, a test of water flux at the imaged level may reveal no water flux difficulty. Second, while cavitation may be an important factor in PD development, because the tests of water conductivity are carried out using water under pressure, cavitated vessels will be re-filled during the test and no reduction in water flux would be revealed. Destructive anatomical work will define which kind of vessel disruption (tylose, gel or air embolism) exists in stems with non-functional vessels as revealed by MRI.

A more quantitative analysis of the MRIs has been attempted in order to characterize objectively the presence of "dark sectors" in the images. For this purpose, the MRIs were processed and analyzed using the ImageJ program (developed at the U.S. National Institutes of Health and available at http://rsb.info.nih.gov/ij). First, the number of functional vessels ($\boldsymbol{N_V}$) was counted in the MRIs of inoculated and control vines (like the one in Figure 9a), based on the assumption that a bright (hence, water-filled) vessel was functional. Next, the xylem-cross sectional area ($\boldsymbol{A_x}$) was measured by isolating in the MRIs (Figure 9b) the ring of tissue that is usually occupied by the xylem. Then, the digital image of the xylem-ring was converted to a binary image (Figure 9c) using a built-in algorithm in ImageJ, in which all the pixels above a set grey intensity threshold are black and the pixels below this value remain white, and the functional xylem-cross sectional area ($\boldsymbol{A_f}$) was determined by measuring the black area. To confirm that the threshold area correctly estimated A_f,,the area of individual functional vessels was selected by hand and measured in a series of MRIs, some with clearly delimited vessel images and others with less distinct ("fuzzy") images such as those often seen when PD-infected grapevine stems are examined. The images from infected vines often do not show vessels as bright or darks spots, rather the images of individual vessels are fuzzy, making determination of vessel functional status difficult. The area of functional xylem measured manually was then correlated with the number of functional vessels (Figure 10), and with the results of the automated routine (Figure11). The regressions confirmed that both the number of functional vessels and the threshold areas depicted in the binary images, are excellent estimators of A_f. Preliminary results of the quantitative analysis described above, in which all the images for an individual plant were averaged; indicate that *Xf*-inoculated vines have a lower mean density of functional vessels (Table 1) than that of controls. Figures 12 and 13 show that the vessel density also correlates positively with the hydraulic conductivity for whole stems, suggesting that the visual assessment of MRIs conveys information about the actual water movement capacity of grapevine stems. Principal components ellipses ($p = 0.5$) in Figures 12 and 13 show that, in both, inoculated and control vines, the hydraulic conductivity for the whole stem is a function of the vessel density, but infected the vines tend to localize

clearly in the lower range of that response. We have shown that cavitated vessels that are air-filled can be re-filled (including restoring an image showing that they are water-filled, see Figure 3). However, attempts at refilling segments of PD-infected stems that showed "dark sectors" in the MRIs generally failed. This indicates that "dark sectors" in MRIs of infected vines are likely a sign of a relatively permanent deterioration of the water movement capacity in the stem, probably a consequence of tylose formation and/or vascular gel development.

Table 1. Mean values for calculated functional vessel densities in healthy and infected grapevine stems.

	Vessel density ± 1 SE	
Treatment	N_V/A_x	N_V/A_f
Control	63.03 ± 4.81	124.88 ±11.93
Xf-inoculation	49.78 ± 4.81	93.25 ±11.93

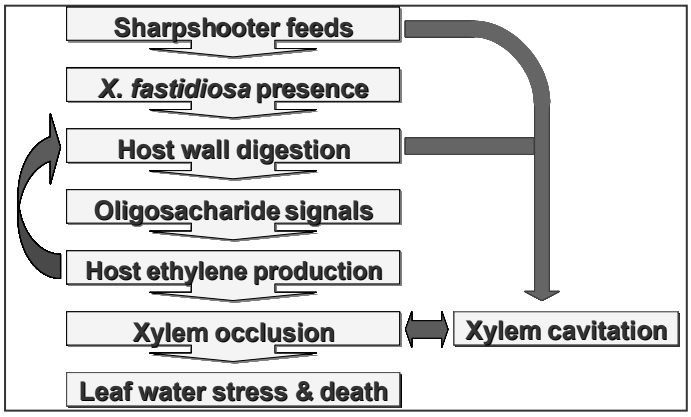


Figure 1. Hypothetical model for PD development. PD starts with a local infection caused by the glassy-winged sharpshooter's introduction of *Xf* locally (i.e., into one or a few vessels). Once *Xf* is in the xylem the bacteria become systemic, which implies that *Xf* must be able to cross (digest away?) the cell wall in the pit membranes that separate two neighboring vessels. The digestion of the cell wall by bacterial enzymes would generate transient oligosaccharides with biological activity. The presence of these oligosaccharides is detected by the plant triggering a series of defensive responses, including a raise in ethylene production. Ethylene has been shown to induce tylose formation. Cavitation of vessels may be also important for the disruption of water transport in the plant. Cavitations may happen during insect feeding or during PD progression. The "bottom line" of our thinking is that PD is primarily caused by the grapevine's responses (local and systemic) to *Xf* presence.

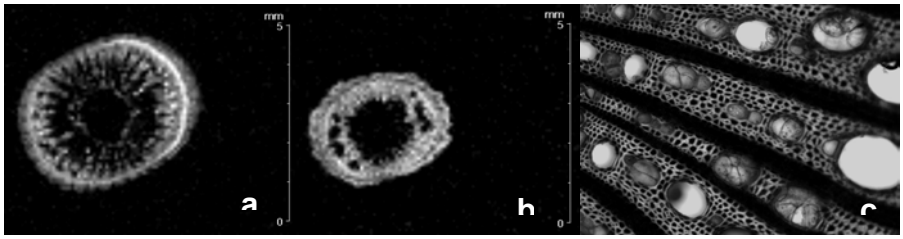


Figure 2. MRI of a PD-infected stem in a basal internode (a), and closer to the apex (b). Bright spots between the central pith (dark) and the ring of vascular cambium show functional vessels. Image b shows dark pockets within the vascular tissue that indicate areas in which vessels are not water-filled (compare the image to the healthy stem in Figure 3a). Tyloses (cellular-physical blockages of the vessels) are often associated with dark spots in MRIs of infected xylem, Tyloses are shown as accumulations of dark, bubble-like structures in vessel seen in the light microscope of an infected stem (c).

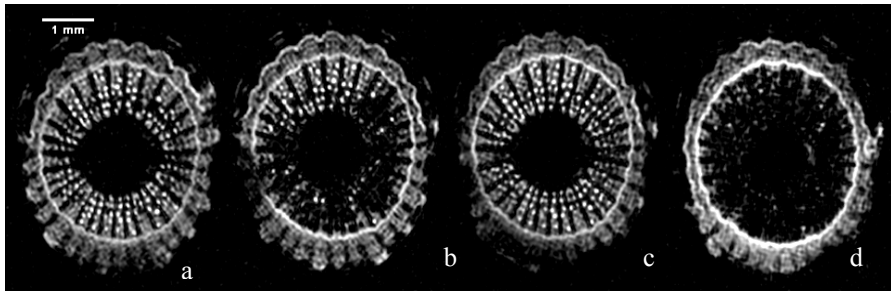


Figure 3. (a) MRI of an intact stem segment in a healthy shoot. (b) Image of the same stem portion after an important part of the cross section below has been severed, thus causing cavitation of many vessels. (c) The same stem segment after it has been refilled with water. (d) Stem segment after flushing with air to completely empty the xylem vessels.

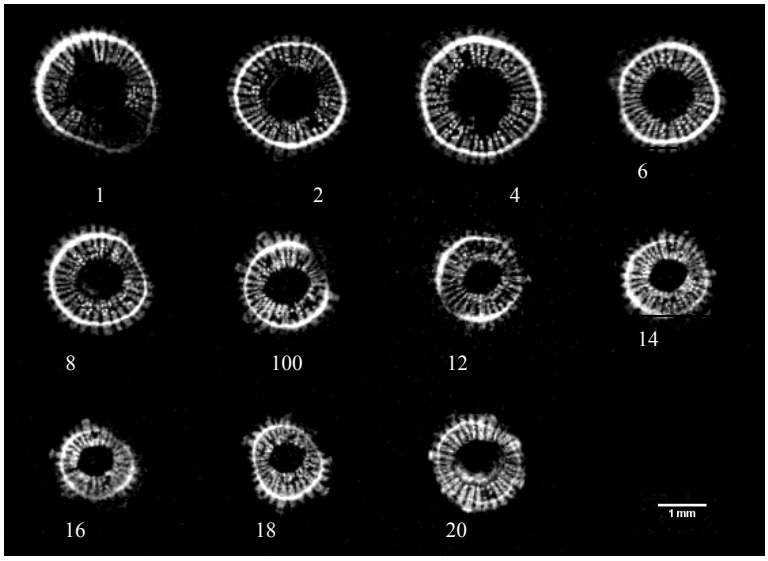
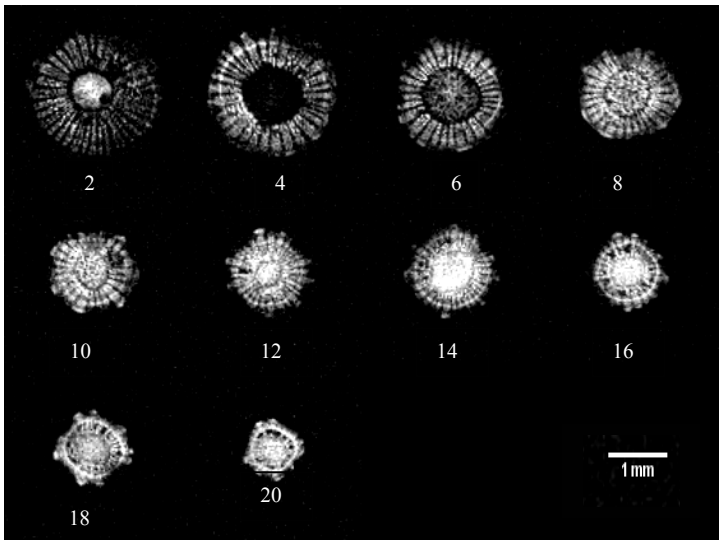


Figure 4. Stem cross section MRIs of a Control (water-inoculated) plant. The numbers indicate the internode position, counting from the base of the stem. In internodes 1-3 it is possible to observe the disruption of the xylem caused by the needle inoculation. The xylem disk looks normal in the other internodes. Note that individual vessels are easily observed as bright spots.

Figure 5. Stem cross section MRIs of an infected plant. This plant was not showing external symptoms after 6 moths of inoculation. The effect of needle inoculation can be seen in internode 2. Dark sectors of embolized vessels can be observed from internodes 10 to 20. Note that in this image it is more difficult to distinguish anatomical features and individual vessel than in MRIs of a Control plant (Figure 4).



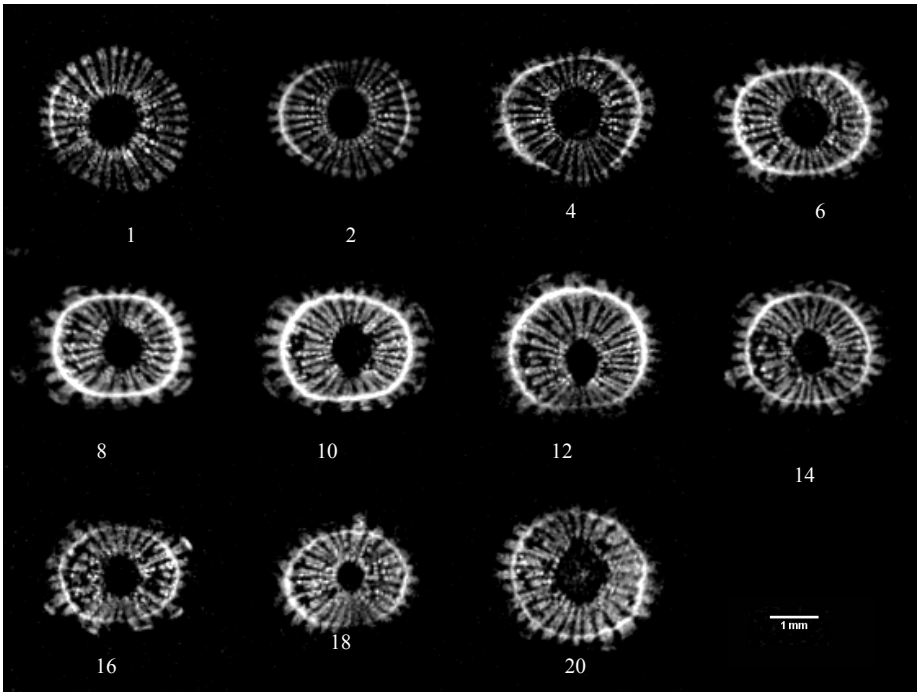


Figure 6. Stem cross MRIs of a plant exposed to ethylene. Numbers indicate the position of the internodes, numbered from the base of the stem. “Dark spots” that show non-functional vessels can be seen increasing in size from the base of the stem. The xylem disk appears to be compromised the most at internode 16, which was approximately the youngest internode in the stem (i.e., in the growing tip) at the time of ethylene treatment.

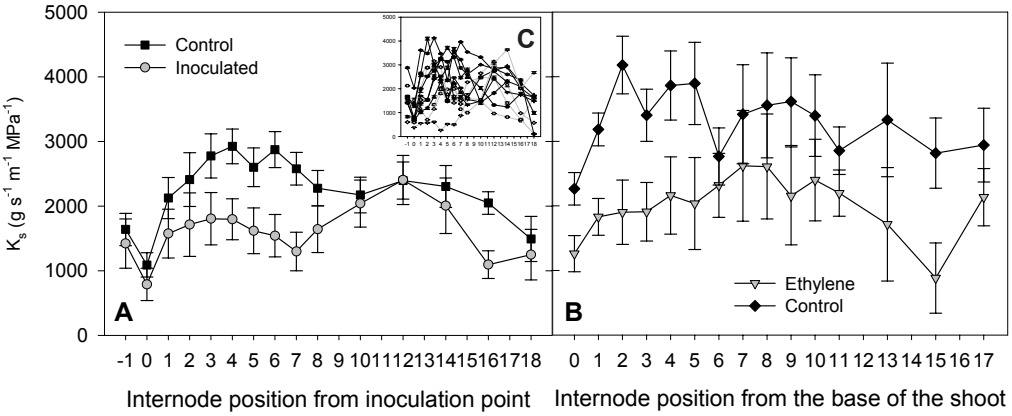


Figure 7. Specific hydraulic conductivities (K_s) for individual internodes of vines (a) inoculated with *Xf* and (b) exposed to ethylene (± 1 SE). Control plants show maximum K_s in middle third of the stem. In contrast, infected plants show a decrease in K_s in the middle portion of the stem. Panel (c) shows K_s ± 1 SD for all the plants analyzed in the inoculation experiment. Although the variation among different plants is high, the error associated with the measurements is negligible. **Note:** These measurements reflect the contribution of water flowing through cavitated vessels because the embolized vessels are filled by the pressurized water that is used in the test.

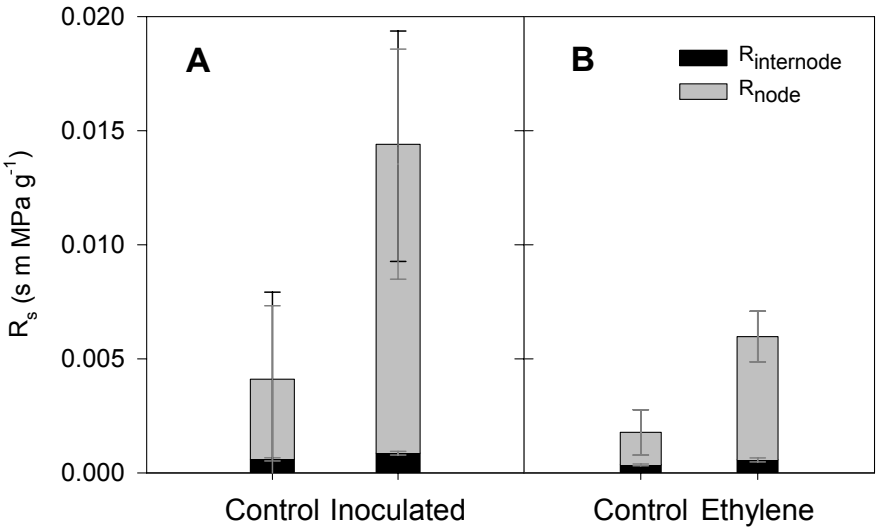


Figure 8. Specific hydraulic resistivity (R_s) for (a) vines inoculated with *Xf* and (b) exposed to ethylene. Total bar height represents R_s ± 1 SE (in black). R_s components, R_{node} and $R_{internode}$, are also shown (± 1 SE in gray). The nodes are a major component of stem hydraulic resistivity (the inverse of conductivity). It can be noted that R_s is about 3 fold higher for stems of infected plants than for controls, even when infected plants have no external symptoms. This observation agrees with the information provided by MRI.

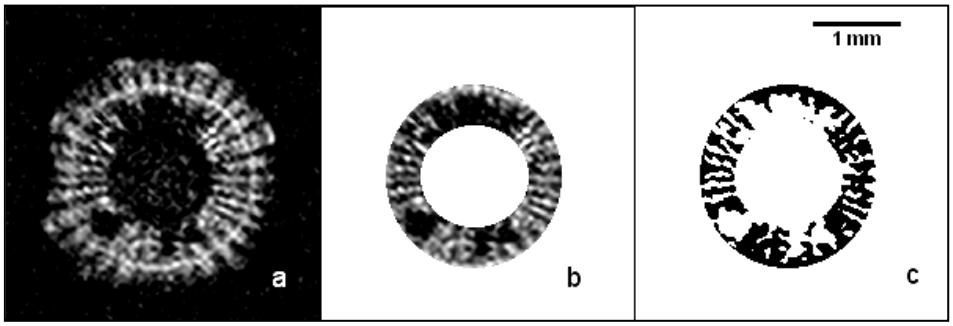


Figure 9. Example of the digital processing and analysis performed on MRIs to evaluate quantitatively the development of dark spots. (a) Original cross section MRI of an infected plant showing dark spots. Individual functional vessels are counted using this type of image. (b) Isolation and quantification of the cross sectional area of the stem that is normally xylem tissue (A_x). (c) Binary analysis of the xylem ring to determine the area of functional xylem (A_f), the black area represents the pixels that are above the threshold defined as the minimum value for a water-filled pixel. The program allows us to vary the threshold value.

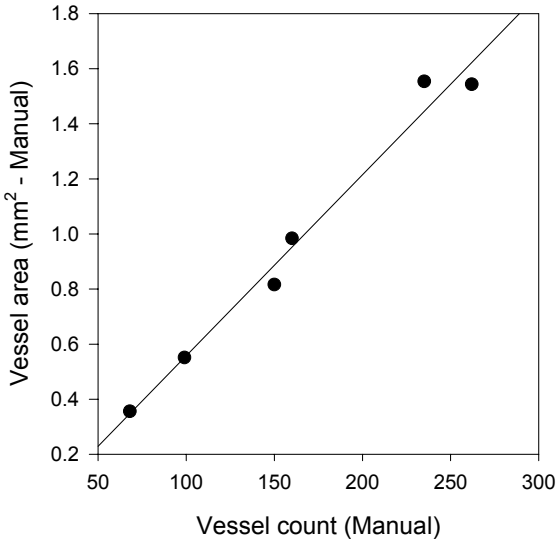


Figure 10. The number of functional vessel (vessel count) is a good predictor of the total area occupied by those vessels. Individual vessel areas were marked on the digitized MRI and summed automatically by ImageJ. Linear regression line $r^2 = 0.98$.

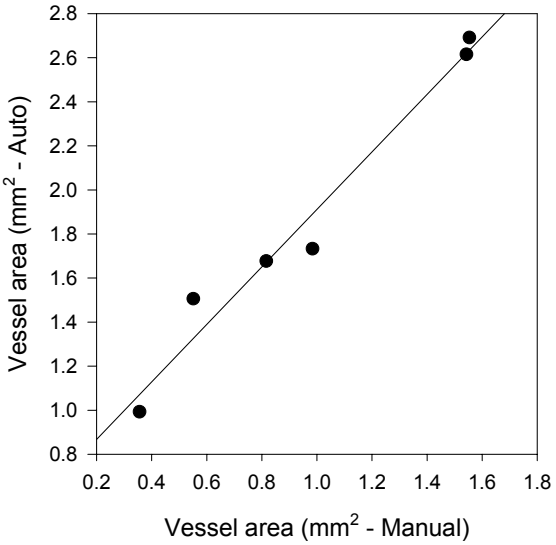


Figure 11. The area of functional xylem (the summation of the areas of individual vessels, see Figure 10 legend) is well correlated with the area calculated using an automated algorithm ($r^2 = 0.97$). A_f is the area calculated using the algorithm.

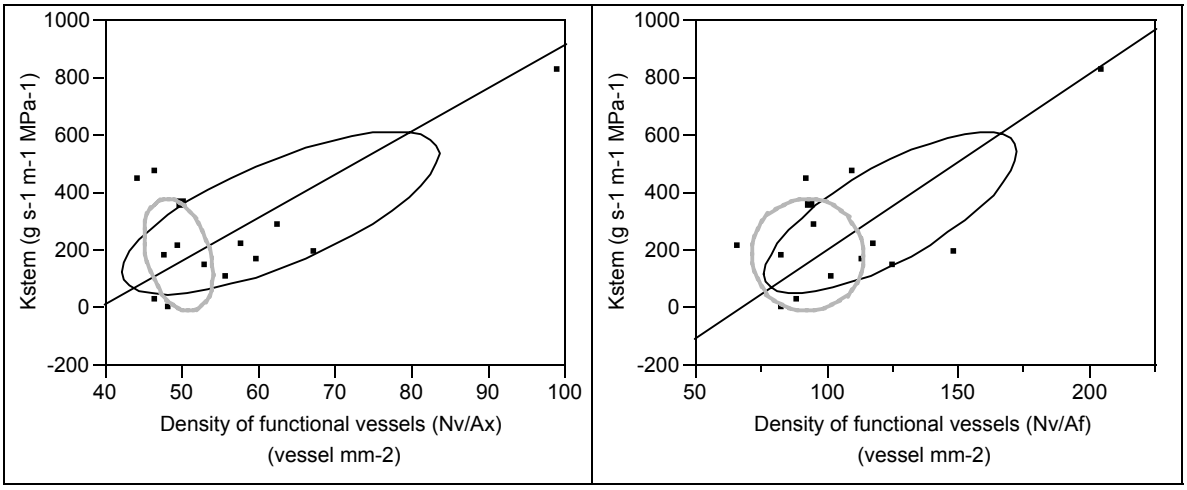


Figure 12. Principal component analysis plotting stem conductivity (y-axis) *vs* functional vessel density calculated as vessel number divided by total xylem area (x-axis). Ellipses enclose values for healthy vines (dashed, light line) and infected vines (heavy, grey line).

Figure 13. As in the Figure 12 legend, except that functional vessel density is calculated as vessel number divided by functional xylem area.

CONCLUSIONS

MRI will be a powerful adjunct to other, more conventional approaches for characterizing the changes that occur in grapevine xylem following introduction of *Xf*.

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FUNDING AGENCIES

Funding for this project was provided by the CDFA Pierce's Disease Research Grant Program.

IMPACT OF HOST PLANT XYLEM FLUID ON *XYLELLA FASTIDIOSA* MULTIPLICATION, AGGREGATION, AND ATTACHMENT

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Reporting Period: The results reported here are from work conducted from October 2003 to August 2004.

ABSTRACT

Research in Temecula Valley indicated that the proximity of citrus groves to vineyards has influenced the incidence and severity of Pierce's disease (PD), *Xylella fastidiosa (Xf)*, in grapes. Although the glassy-winged sharpshooter (GWSS) feeds on and moves back and forth between Temecula citrus groves and vineyards, there are no visible *Xylella fastidiosa (Xf)* symptoms in the citrus. This implies that citrus trees are resistant or tolerant to the *Xf* but may be a reservoir to harbor the pathogen for GWSS acquisition while grape vines are susceptible. We investigated the mechanisms of host plant resistance/susceptibility by examining the impact of xylem fluid of grapefruit, orange, lemon and grape on *Xf* multiplication, aggregation and attachment as well as the related xylem fluid chemistry. Our laboratory experiments revealed that xylem fluid of grapefruit, orange and lemon caused an aggregation of Temecula PD cells to form large white clumps while grape xylem fluid did not cause visible clumping, but created a visible thick biofilm. The numbers of *Xf* cells in grapefruit xylem fluid treatment were significantly higher at 6, 8 and 9 days after culture compared with those in grape xylem fluid treatment. The numbers of *Xf* cells in orange or lemon xylem fluid tests were generally lower than those in grape xylem fluid treatment. Citrus xylem fluid significantly inhibited *Xf* biofilm formation compared to grape xylem fluid. The content of total amino acids in grape xylem fluid was near 9-fold higher than that in grapefruit xylem fluid. Sugar contents were 1.4- to 5.5-fold higher in grape xylem fluid than those in grapefruit xylem fluid. Peroxidase and total thiol levels were also higher in grape xylem fluid than in citrus xylem fluid. Our results indicate that the differences between citrus and grape plants in their responses to *Xylella* may be due to differences in their xylem fluid chemistry.

INTRODUCTION

Xylella fastidiosa (*Xf*) is a xylem-limited, plant pathogenic bacterium that causes Pierce's disease (PD) in grapes (Purcell, 1981). *Xf* is mainly vectored by the glassy-winged sharpshooter (GWSS), *Homalodisca coagulata,* in Southern California. Although a comprehensive list of suitable hosts for the GWSS has been identified, comprising 75 plant species in 35 families (Turner and Pollard, 1959), the major crop hosts in Temecula Valley are citrus and grapes. Previous studies in California have identified 94 plant species in more than 28 of plant families as host of *Xf* (Freitg, 1951; Raju et al, 1983; Raju et al., 1980). Most identified *Xf* hosts show no symptoms but serve as inoculum sources of *Xf* for vector acquisition. Perring et al (2001) studied the incidence of PD in the Temecula Valley and found that proximity of citrus groves to vineyards has influenced the incidence and severity of PD in grapes. The PD infection is most severe when the grape vines are adjacent to citrus, and that the damage declines as one moves away from citrus (Perring et al., 2001). Although the GWSS feeds on and moves back and forth between citrus trees and grape vines, there is generally no *Xf* caused disease symptom in citrus in the area. This implies that citrus trees are resistant or tolerant to the *Xf*, but may be a reservoir to harbor the pathogen for GWSS acquisition and transmission while grape vines are susceptible. Little is known about the biochemical mechanisms involved in host plant resistance/susceptibility to *Xf* in the system. Additional information is required to determine if citrus can be suitable reservoirs for *Xf*. Elucidation of the biochemical mechanisms may be useful for developing host plant resistance in grapes as a sustainable component of integrated pest management program.

Xf aggregates to form biofilm inside its host plants and insect vectors. The biofilm formation is considered as a major virulence factor of PD (Marques and Ceri, 2002). Biofilm is defined as structured communities of sessile microbial aggregates enclosed in a self produced polymeric matrix and attached to a surface (Costerton et al., 1995). It was recently reported that a defined medium with some components based on susceptible grape cultivar "Chardonnay" xylem fluid chemistry better supports *Xf* growth and stimulates *Xf* aggregation and biofilm formation in vitro (Leite et al. 2004). However, the effect of citrus xylem fluid on *Xf* multiplication, aggregation and biofilm formation remains unknown.

Xf is a nutritionally fastidious bacterium (Wells et al. 1987). In defined medium certain amino acids are essential for *Xf* growth, glucose stimulates the growth while fructose and sucrose have inhibiting effect (Wells et al. 1987; Chang and Donaldson, 2000). It is not known whether differences in contents of amino acids and the sugars in the xylem fluid of citrus

and grape may differentially affect growth of *Xf*. Redox status also likely affects the tendency for *Xf* aggregation and biofilm formation. Adding reducing agents such as glutathione to artificial medium promotes *Xf* aggregation and biofilm formation (Leite et al., 2004). It was reported that thiols mediate the aggregation and adhesion of *Xf* (Leite et al., 2002). Thiol-containing compounds in xylem fluid include cysteine, methionine and glutathione. The redox status in citrus and grape xylem fluid and its role in *Xf* aggregation and biofilm formation, and host plant resistance/susceptibility to *Xf* need to be further investigated.

OBJECTIVES

1. Investigate the effect of host plant xylem fluid on *Xf* multiplication, aggregation and attachment.
2. Determine the biochemical mechanisms of host xylem fluid influence on *Xf* multiplication, aggregation and attachment.

RESULTS

Commercial citrus (lemon, orange and grapefruit) groves in proximity to vineyards were selected in the Temecula Valley, California. Three blocks of 30 citrus and 30 grape vines were used. A minimum of 15 citrus trees and 15 vines were randomly selected from each block (making a total of 15 trees or vines from each plant species) to extract xylem fluid. Terminal shoots from each plant were used for xylem extraction with a pressure bomb apparatus (Anderson et al., 1989). Upon collection, the xylem fluid was immediately placed on dry ice before final storage in a -80 ^{o}C freezer. The samples were used to test the impact of these xylem fluid on *Xf* resistance and chemical analyses of soluble carbohydrates, free amino acids, and redox status.

Effects of xylem fluid of each plant species on *Xf* attachment were evaluated on the biofilm formation. Formation of biofilm on the abiotic surfaces was assessed as described by Espinosa-Urgel et al. (2000). The analyses of *Xf* multiplication and aggregation were based on the fact that optical density (540 nm) is correlated with bacterial cell numbers and aggregation state as described by Burdman et al. (2000).

Our data indicated that, when the xylem fluid of grapefruit, orange and lemon was added to the PD Temecula strain of *Xf* in PD3 medium in glass culture tubes, there were heavy *Xf* cell aggregations to form large white clumps in suspension of the culture and the culture fluid was clear with no significant turbidity; in contrast, grape xylem fluid added to the same *Xf* culture did not cause visible clumping, but rather a visible thick biofilm was formed on the surface of glass tube and the culture was turbid (Figure 1). After homogenization of the culture, we found that the numbers of *Xf* cells in the grapefruit xylem fluid treatment were significantly higher at 6, 8 and 9 days after culture compared with those in the grape xylem fluid treatment (Figure 2). The numbers of *Xf* cells in orange or lemon xylem fluid treatments were generally lower than those in grape xylem fluid treatment (Figure 3). These data suggest that the citrus species, especially grapefruit, are suitable hosts for *Xf* growth and may serve as a great reservoir of the pathogen for GWSS acquisition. Our assay results revealed that xylem fluid of the citrus species significantly inhibited *Xf* biofilm formation compared to that of grape (Figure 4). Our attempt to investigate the biochemical mechanisms likely to be involved indicated that 96% of amino acids in grape xylem fluid was comprised of glutamine, while 47% of amino acids in grape fruit xylem fluid was proline (Figure 5). The content of total amino acids in grape xylem fluid was near 9-fold higher than that in grapefruit xylem fluid (Figure 5). Sugar contents were 1.4- to 5.5-fold higher in grape xylem fluid than those in grapefruit xylem fluid (Figure 6). Peroxidase and total thiol levels were also higher in grape xylem fluid than in citrus xylem fluid (Figures 7 and 8).

CONCLUSIONS

Xylem fluid of grapefruit, orange and lemon caused PD Temecula strain of *Xf* cells to aggregate and form large white clumps but inhibited the attachment. In contrast, grape xylem fluid did not cause visible clumping but led to heavy attachment. Grapefruit xylem fluid significantly increased multiplication of *Xf* cells compared with grape xylem fluid. Citrus species, especially grapefruit, appear to be suitable hosts for *Xf* growth and may serve as a reservoir of the pathogen for GWSS acquisition and transmission to grape vines. Further research is underway to elucidate the biochemical mechanisms.

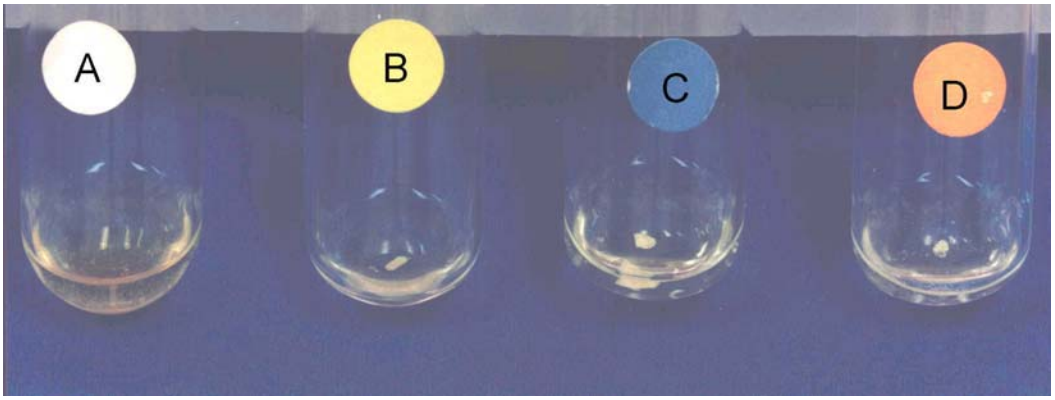

Figure 1. Effect of host plant xylem fluid on *Xf* aggregation. A, treatment with grape xylem fluid. B, treatment with grapefruit xylem fluid. C, treatment with orange xylem fluid. D, treatment with lemon xylem fluid. Note that white clumps of *Xf* aggregates are formed in the grapefruit, orange and lemon xylem fluid treatments.

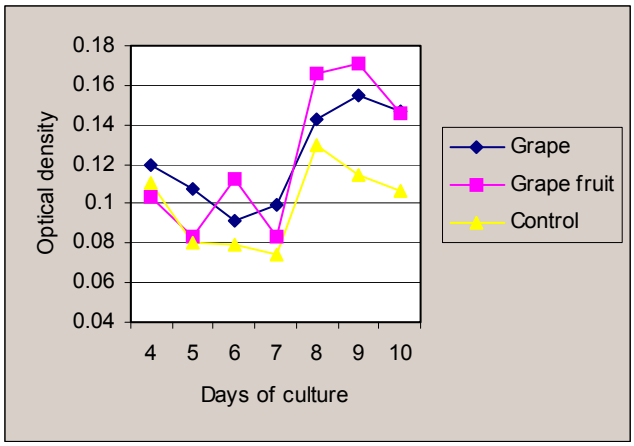

Figure 2. Effect of host plant xylem fluid on *Xf* growth.

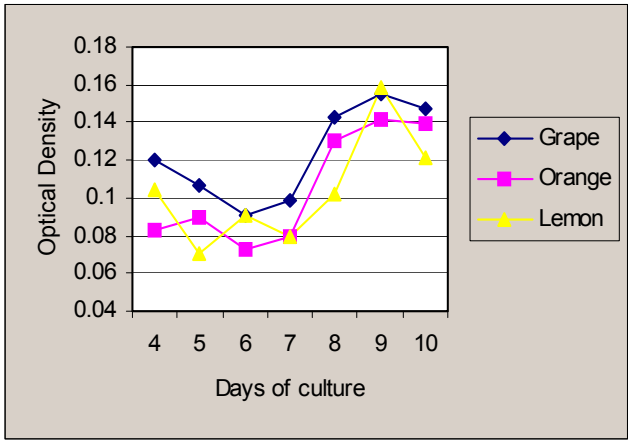

Figure 3. Effect of host plant xylem fluid on *Xf* growth.

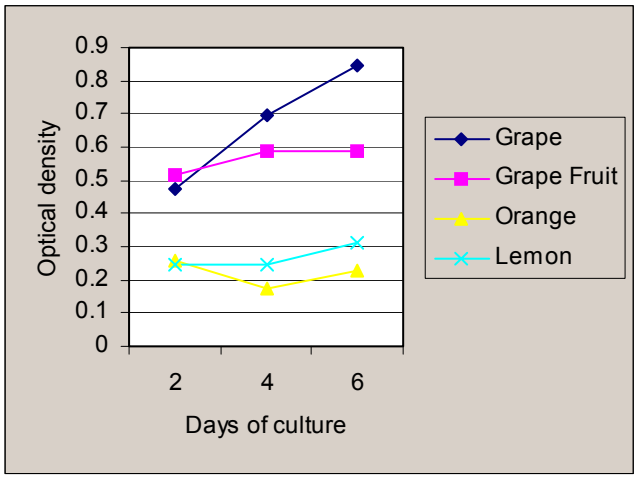

Figure 4. Effect of host plant xylem fluid on *Xf* biofilm formation.

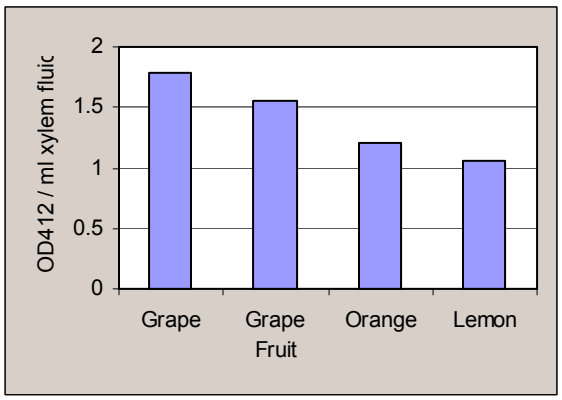

Figure 8. Total thiol contents in host xylem fluid.

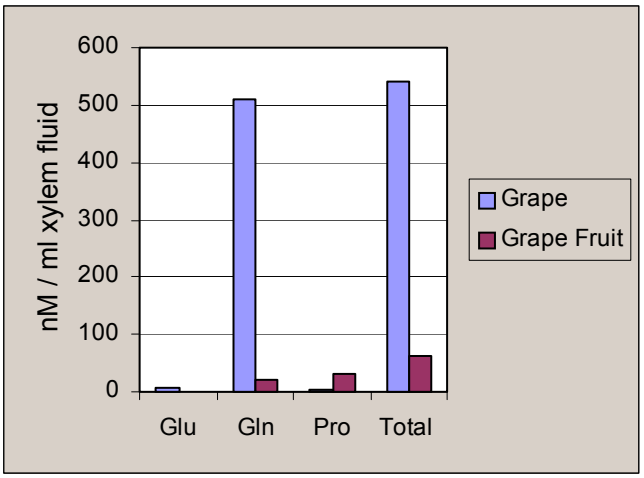

Figure 5. Some amino acid contents in grape and grape fruit xylem fluid.

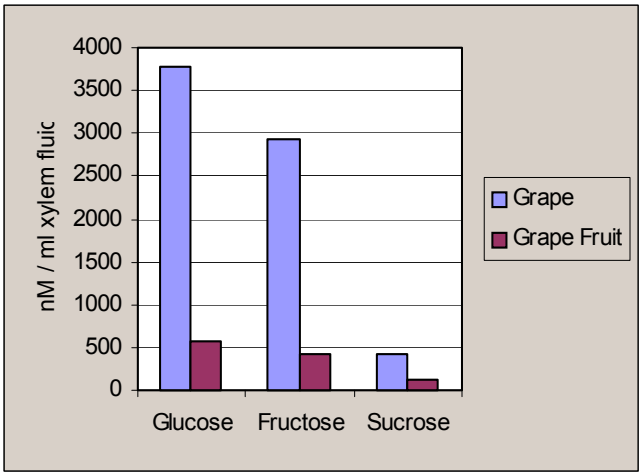

Figure 6. Sugar contents in grape and grape fruit xylem fluid.

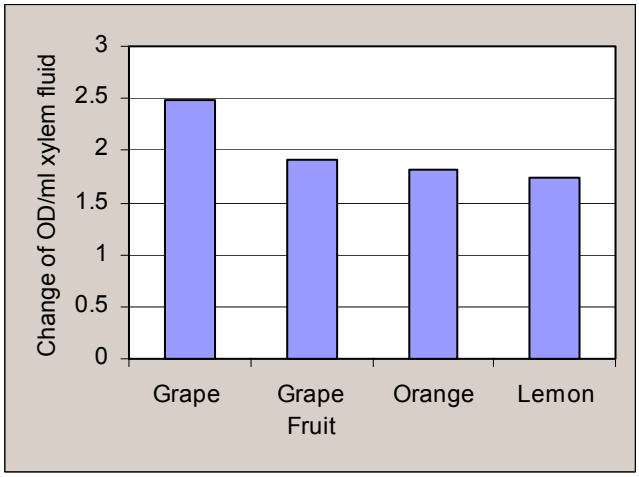

Figure 7. Peroxidase levels in host xylem fluid.

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FUNDING AGENCY

Funding for this project was provided by the University of California Pierce's Disease Grant Program.

OPTIMIZING MARKER-ASSISTED SELECTION FOR RESISTANCE TO *XYLELLA FASTIDIOSA* TO ACCELERATE BREEDING OF PIERCE'S DISEASE RESISTANT GRAPES OF HIGH FRUIT QUALITY

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Reporting Period: The results reported here are from work conducted from October 2003 to October 2004. Research on this project was initiated under the "Genetics of Resistance to Pierce's Disease" of the Long-term American Vineyard Foundation Pierce's Disease Project.

ABSTRACT

Efforts at identifying molecular markers linked to *Xylella fastidiosa* (*Xf*) resistance are continuing. Our primary focus is on resistance derived from b43-17, a *Vitis arizonica/candicans* type collected near Monterrey, Nuevo Leon, Mexico. The '9621' *V. rupestris* x *V. arizonica* hybrid mapping family (PD resistant D8909-15 x PD resistant F8909-17) was used to localize *PdR1*, a primary PD resistance locus within the linkage map of the male parent F8909-17 (progeny of b43-17) and identify candidate linked resistance markers. In more recent research, a comparative mapping strategy between the '9621' linkage map and other SSR maps within *Vitis* was used to identify 9 SSR markers within 10 cM of the resistance locus. Resistance from the female parent D8909-15 has not yet been localized to a genetic map. The strategy of bulk segregant analysis (BSA) in concert with the AFLP marker system has been initiated to saturate the region around the resistance locus and is expected to yield an additional 20 to 50 markers linked to the resistance trait. All candidate resistant markers have been and will continue to be applied to breeding populations derived from '8909' x *V. vinifera* and ('8909' x *V. vinifera*) x *V. vinifera* back-cross generations in order to confirm resistance marker effectiveness in *V. vinifera* backgrounds and continue with marker assisted selection for development of high quality PD resistant grapes.

INTRODUCTION

Several American *Vitis* species are native to the regions where PD is endemic, and resistance from these sources has been introgressed into many different cultivars grown in the south-eastern United States. The acceptance of the new hybrid cultivars has been limited due in part to some undesirable non-vinifera fruit quality traits. The development of high quality PD resistant cultivars will be facilitated by the use of molecular markers to achieve a more precise introgression of the resistance genes into domesticated backgrounds and avoid introgression of undesirable traits (Figure 1). Backcross introgression via molecular markers has been accomplished successfully in other crops (Young and Tanksley 1989). This type of introgression is generally termed Marker Assisted Selection (MAS), whereby indirect selection on a trait of interest (such as disease resistance) is made by screening for the presence of a DNA marker allele tightly linked to the trait. MAS for disease resistance can also be used to eliminate susceptible genotypes in a breeding population early in the selection process, which allows for evaluation of much larger effective populations. Larger effective population sizes increase the opportunity to identify genotypes with high disease resistance and good horticultural qualities (such as good flavor traits, color, berry and cluster size. etc.). Other key aspects of the MAS process include avoiding confounding environmental effects on the trait phenotype and accelerating breeding progress while saving space and time, allowing for more efficient use of resources (Paterson et al. 1991, Kelly 1995). Rapid screening time is particularly valuable when applied to perennial crops such as grape with relatively long generation times (Alleweldt 1988, Striem et al. 1994). To effectively use linked markers in MAS only requires that the markers be highly reproducible, linked in coupling phase i.e. on the same homologous chromosome, and within 5 centimorgan (cM) mapping units of the resistance locus (Kelly 1995).

Within grapevines, markers linked to powdery mildew resistance (Dalbo et al. 2001, Pauquet et al. 2001), downy mildew resistance (Luo et al. 2001) and seedlessness (Lahogue 1998) have been published. In the case of powdery mildew resistance, MAS has already been successfully utilized for screening a grape breeding population. We are successfully developing a MAS system for screening PD resistant genotypes that will greatly benefit our breeding of PD resistant wine grapes.

OBJECTIVES

Our overall objective is to identify DNA markers that are tightly linked to the primary locus or loci required for complete resistance to PD within *Vitis*. Research will focus on PD resistance as inherited from *V. arizonica* and will utilize an established *V. rupestris* x *V. arizonica* genetic map. These markers will be utilized for MAS to eliminate susceptible seedling progeny our continuing PD resistance breeding program.

Sub-objectives

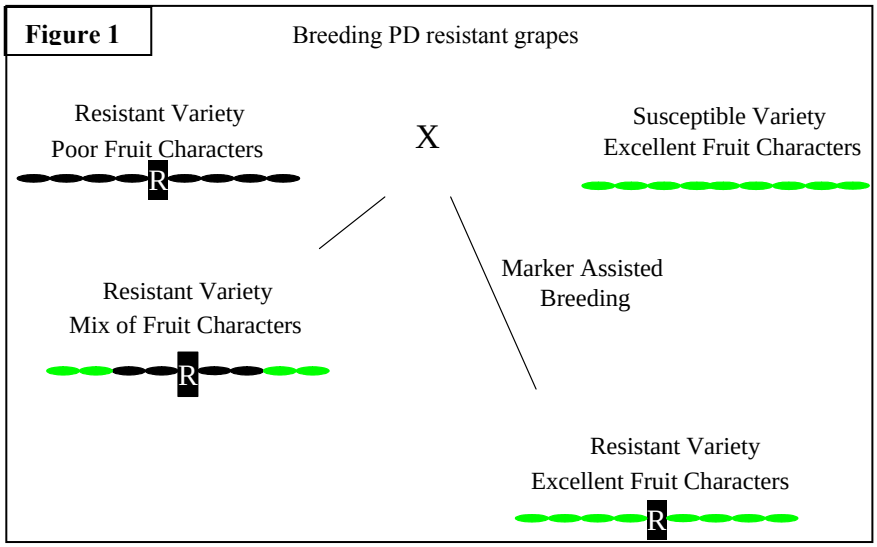
1. Continue with a comparative mapping strategy between the *V. rupestris* x *V. arizonica* 9621 (D8909-15 x F8909-17) linkage map and other SSR maps within *Vitis* in order to identify additional SSR markers linked to resistance.

2. Utilize Bulk Segregant Analysis (BSA) with the AFLP marker system to saturate with markers the region around the previously mapped *Xf* resistance locus and eventually convert confirmed candidate markers to stable SCAR primers.
3. Confirm candidate marker linkage to resistance within families derived from resistant by susceptible crosses such as the '8909' x *V. vinifera* and ('8909' x *V. vinifera*) x *V. vinifera* back-cross generations.

RESULTS AND CONCLUSIONS

Sub-objective 1.

Initial mapping of the PD resistance locus *PdR1* in the male parent F8909-17 of the 9621 family localized it to chromosome 14, and identified 6-8 SSR markers on the same linkage group. Marker placement on published SSR linkage maps of *Vitis* were used to preferentially target chromosome 14, bringing the total number of SSR markers on the linkage group up to 30. Approximately 9 SSR markers are localized within a 10 cM distance of the resistance gene. These SSR markers are reliable and are the easiest of the molecular markers to incorporate within a MAS breeding program. Correlation tests of these candidate markers to PD resistance when functioning within a *V. vinifera* genetic background are underway and described in sub-objective 3. The SSR marker analysis has allowed us to confirm that marker alleles linked in coupling to PD resistance alleles of the *PdR1* locus in another PD resistant progeny of b43-17 (F8909-08) are different than the alleles linked in coupling the resistance alleles in F8909-17. It is apparent from these results that b43-17 is homozygous resistant for the PdR1 locus, and that F8909-17 inherited its resistance allele from one chromosome 14 and F8909-08 inherited its resistance allele from the homologous chromosome 14. In either case the markers linked to resistance will function for MAS, however, different alleles linked in coupling to the resistance alleles will have to be followed through the downstream MAS process. Placement of SSR markers to chromosome 14 via the comparative mapping strategy continue as the markers become available, however, the number of SSR markers that can be targeted to a specific chromosomal region via comparative mapping is limited.



Sub-objective 2.

For high density marker saturation within a narrow window around the *PdR1* locus, a bulk segregant analysis (BSA) strategy (Michelmore et al. 1991) in concert with the AFLP marker system was chosen as the method of choice. Initial BSA was attempted within the 9621 family, however, confounding effects of the resistance loci within the D8909-15 parent made the attempt more difficult than expected. To avoid confounding affects from resistance inherited from other genetic backgrounds and focus the BSA procedure only on the *PdR1* locus, work has begun within two segregating families from susceptible by resistant crosses. The first family, 99217 (C8909-07 x F8909-08) consists of 33 genotypes, has been screened for PD resistance (Krivanek et al. submitted) and segregates 1:1 resistant to susceptible (Table 1). DNA has been extracted from these genotypes, flanking SSR markers were run and a good correlation between resistance and resistance marker alleles has been established (Table 1). A bulk of the DNA from the 12 most susceptible and a bulk of the DNA from the 12 most resistant genotypes are in process and will be tested for AFLP polymorphisms utilizing florescent primers and visualized on a PE 3100 sequencer. The second family derived from a susceptible by resistant cross is a *V. vinifera* x F8909-08 family; it consists of 40 genotypes and has been designated as 0062. Testing of this family for PD resistance is currently underway via our standard greenhouse testing procedure (Krivanek et al. in press; Krivanek and Walker in press). It is expected that the progeny in this family will segregate in a 1:1 manner, and if so, DNA extraction and BSA procedures will be undertaken as with the 99217 family. Candidate AFLP markers will be converted to stable and more reliable SCAR primers before incorporation into the MAS program.

Sub-objective 3.

Work is progressing with two distinct breeding populations for testing of candidate resistance markers and initial application of those markers to MAS. One family is a cross of the PD resistant F8909-08 to a female *V. vinifera* wine grape F2-7 (Cabernet Sauvignon x Carignane) and designated as the 0062 family. A second breeding population consists of a cross of F8909-08 to several elite *V. vinifera* table grape genotypes (the 500 series). A subset of the 500 series has been screened for PD resistance and screened for markers flanking the *PdR1* locus. Five confirmed resistant genotypes have been utilized in the development of the first backcross generations BC1 (backcrossed to additional elite *V. vinifera* genotypes). The BC1 population (25000 series) consists of approximately 200 individuals and was planted in the field in 2003. Marker analysis for flanking markers to the *PdR1* locus has been completed for the 25000 series and the marker information was utilized in selection of genotypes for the spring of 2004 crosses for the development of the BC2 generations. Subsets of candidate

resistant and susceptible genotypes within the 25000 series have shown improved fruit quality (Figure 2) and are currently being screened to confirm the correlation between the resistance markers and the PD resistance trait. We are also utilizing these populations to confirm the effectiveness and economics of the MAS relative to our greenhouse screening procedure.

Table 1. Resistance classification and marker genotypes for the individuals of the full-sib family derived from the susceptible by resistant cross of C8909-07 x F8909-08. * = Genotypes selected for Bulk Segregant Analysis procedure.

Genotype	Overall resistance level to PD	Mean natural log (cells/ml)	Mean CMI score	Mean % leaf scorch	Alleles of SSR markers flanking the PdR1 resistance
99217-21 *	Resistant	9.51	1.00	58.3	Rr / Rr
99217-40 *	Resistant	9.70	1.33	75.0	rr / Rr
99217-18 *	Resistant	9.77	2.75	95.0	Rr / Rr
99217-41 *	Resistant	10.19	4.25	76.3	Rr / Rr
99217-35 *	Resistant	10.55	1.33	100.0	rr / Rr
99217-19 *	Resistant	11.08	2.50	76.7	rr / Rr
99217-01 *	Resistant	11.52	2.25	90.0	rr / Rr
99217-23 *	Resistant	11.57	3.00	87.5	Rr / Rr
99217-34 *	Resistant	11.83	3.75	65.0	Rr / Rr
99217-46	Resistant	11.87	5.75	100.0	Rr / Rr
99217-27 *	Resistant	12.20	4.25	100.0	Rr / rr
99217-22 *	Resistant	12.29	4.00	100.0	Rr / Rr
99217-12 *	Resistant	12.50	4.00	95.0	Rr / Rr
99217-38	?	12.69	5.00	100.0	Rr / Rr
99217-36	?	13.09	5.00	100.0	rr / rr
99217-50	?	13.52	4.25	83.8	Rr / Rr
99217-14	Susceptible	14.06	5.50	88.8	rr / Rr
99217-07	Susceptible	14.87	5.50	100.0	rr / rr
99217-04 *	Susceptible	15.42	6.00	100.0	rr / rr
99217-33 *	Susceptible	15.59	5.75	100.0	rr / rr
99217-06 *	Susceptible	15.80	5.25	68.3	rr / rr
99217-09 *	Susceptible	15.81	5.75	100.0	rr / rr
99217-10	Susceptible	15.82	4.75	100.0	rr / rr
99217-13 *	Susceptible	15.84	5.50	100.0	rr / rr
99217-42	Susceptible	15.85	4.25	75.0	rr / Rr
99217-15 *	Susceptible	15.87	5.25	100.0	rr / rr
99217-32 *	Susceptible	15.87	5.50	100.0	rr / rr
99217-28 *	Susceptible	15.91	5.75	100.0	rr / rr
99217-05 *	Susceptible	15.91	5.75	100.0	rr / rr
99217-37 *	Susceptible	15.92	5.25	100.0	rr / rr
99217-26 *	Susceptible	15.95	5.50	100.0	rr / rr
99217-24 *	Susceptible	16.04	6.00	100.0	rr / rr

Figure 2.

Vitis arizonica PD Resistant poor fruit quality

Hybrid BC1-25017 with flanking PD resistance markers Improved fruit quality

Vitis vinifera PD Susceptible Excellent fruit quality

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FUNDING AGENCIES

Funding for the 2004-2005 funding year was received in mid-September 2004. This proposal was not submitted to other funding agencies. However, it is linked to the Walker/Tenscher Pierce's disease resistance breeding project funded by the CDFA Pierce's Disease and Glassy-winged Sharpshooter Board (and formerly by the California Table Grape Commission and the California Raisin Advisory Board), and the Walker/Riaz mapping project. This project was initiated through funding by the American Vineyard Foundation and CDFA for the Genetics of Resistance to Pierce's disease, a project that developed a framework map for the 9621 population. Funding from the Louis P. Martini Endowed Chair in Viticulture has also supported Pierce's disease mapping and marker development projects.

MAP BASED IDENTIFICATION AND POSITIONAL CLONING OF *XYLELLA FASTIDIOSA* RESISTANCE GENES FROM KNOWN SOURCES OF PIERCE'S DISEASE RESISTANCE IN GRAPE

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Reporting Period: The results reported here are from work conducted from November 2003 to October 2004.

ABSTRACT
Development of an SSR genetic linkage map based on the 9621 family is continuing. The family segregates for PD resistance and is based on the cross of PD resistant D8909-15 x PD resistant F8909-17. We expanded the mapping population size from 116 to 188 genotypes. The current genetic linkage map consists of 217 non-AFLP markers (SSR, EST-SSR and ESTP) in 19 linkage groups. The PD resistance locus *PdR1* maps to linkage group 14 of the male parent (F8909-17), which now consists of 30 markers, 9 of which are localized within 10 cM of *PdR1*. To avoid confounding affects from resistance inherited from D8909-15 additional families derived from a susceptible by resistant cross are currently being evaluated for map based cloning of the *PdR1* locus. A family from the cross of F2-7 (a cross of two *V. vinifera* wine grapes, Cabernet Sauvignon x Carignane) x F8909-08 (a PD resistant sibling of F8909-17) has been made and is currently being screened for PD resistance via our standard greenhouse testing procedure. To saturate a narrow region around the resistance locus with molecular markers, bulk segregant analysis (BSA) in concert with the AFLP marker system has been initiated in cooperation with our report titled "Optimizing marker-assisted selection (MAS) for resistance to *Xylella fastidiosa* to accelerate breeding of PD resistant grapes."

INTRODUCTION
This project expands upon and continues a genetic mapping effort initiated with funding from the California Grape Rootstock Improvement Commission, the Fruit tree, Nut tree and Grapevine Improvement Advisory Board, the California Table Grape Commission and the American Vineyard Foundation. The project has been mapping resistance to *Xiphinema index*, the dagger nematode, and *Xylella fastidiosa* (*Xf*) in an "F2" population designated as the 9621 family (D8909-15 x F8909-17). A genetic map of 116 individuals from the 9621 population was created primarily with AFLP markers (Doucleff et al. 2004). Our efforts were expanded to informative markers, such as microsatellites or simple sequence repeats (SSR) for two main reasons. First, a genetic map based on SSR markers provides a reliable and repeatable framework for initial mapping of candidate genes and quantitative trait loci (QTLs). Secondly, SSR markers tightly linked to resistance and phenotypic traits of interest are ideal for marker-assisted selection due to their applicability across different genetic backgrounds and ease of use. The grape genetic research community formed the International Grape Genome Program (IGGP) to increase coordination and cooperation and to enhance knowledge of the grape genome. Use of the SSR marker system is common among the different research groups so that our mapping efforts can be linked to others. Integrating the 9621 genetic linkage map to other mapping populations will facilitate targeting genomic regions that harbor quantitative trait loci. Comparison to other maps will allow us to identify more markers that are linked to *Xf* resistance and optimize marker-assisted selection strategies applied to breeding programs. For fine scale mapping a narrow region around the primary resistance locus, we include procedures here. The proposal will expand to include construction and utilization of a genomic library of a resistant parental genotype for eventual cloning of the PD resistance gene.

OBJECTIVES
1. Increase the base population from 116 to 188 genotypes within the 9621 family and expand to a family based on a susceptible by resistant cross of 2,000 to 4,000 genotypes.
2. Increase the number of SSR and EST markers on the core genetic linkage map from 100 to 300 markers.
3. Screen an additional 100-150 EST derived SSR markers for which functions are known after their comparison to homologues in available EST databases.
4. Develop core framework map with an average distance of 2 to 5 cM between markers and utilize Bulk Segregant Analysis (BSA) with the AFLP marker system to saturate a 1 cM region around the *PdR1* resistance locus.

RESULTS AND CONCLUSIONS
Objective 1
The original starting material for this project was a molecular marker linkage map of the 9621 population based on 116 individuals (Doucleff et al. 2004). We expanded the core set of individuals from the 9621 to 188 genotypes to take advantage of 96-well plate based techniques and to increase resolution on the map to improve marker association with PD resistance. A second family derived from a susceptible by resistant cross of F2-7 (a *V. vinifera* wine grape, Cabernet Sauvignon x Carignane) x F8909-08 (a PD resistant sibling of F8909-17) has been made, and 40 individuals are currently being screened for PD resistance via our standard greenhouse testing procedure. An expansion of the family was made in the

Spring 2004 and a total of 4,500 seeds have been collected and placed into cold stratification. Should the initial subset of the family segregate in a 1:1 resistant to susceptible ratio as expected the expanded family of approximately 2,000 to 3,000 genotypes will be an excellent choice for fine resolution placement of the *PdR1* resistance gene. This would be the first step toward placement of resistance markers (flanking the *PdR1* locus) onto a bacterial artificial chromosome (BAC) within a genomic library in a procedure termed "chromosome landing" (Tanksley et al. 1995). Plans for construction of the library are underway.

Objective 2

The original genetic linkage map was based primarily on AFLP markers with 375 placed on the map, with an additional 32 ISSR, 25 RAPD and 9 SSR markers (Doucleff et al. 2004). Our efforts expanded to more reliable SSR markers in order to construct a repeatable framework map useful for more precise placement of primary resistance genes, QTL analysis and marker-assisted selection. Among the marker classes added to the map 310 SSR markers have been tested, 155 were polymorphic in the parents and all have been added to the map; 90 EST derived SSR markers have been tested, 60 of them were polymorphic and 46 have been added to the map; 20 EST markers (provided by Doug Adams) have been tested and 16 were added to the map (Table 1). A total of 217 markers (SSR, EST-SSR and ESTP) tested on 188 genotypes have now been utilized for map construction.

The 217 SSR markers included some that have been previously published and many that were developed by Vitis Microsatellite Consortium and are as yet unpublished. All markers were tested on a small set of 8 DNA samples including both parents and run on 6 % polyacrylamide gels. DNA on the gels was visualized by silver staining with a commercial kit (Promega). We have tested and used all available informative genomic microsatellite markers for the 9621 population. Meanwhile, we also initiated collaboration efforts with the research group at INRA (Montpellier, France) to obtain primer sequences of SSR markers developed at their facility.

To develop ESTP (expressed sequence tagged polymorphism) markers, sequences of grape cDNA were obtained from Dr. Doug Adams (Department of Viticulture and Enology, UC Davis). Potential PCR primers were designed using the computer program PRIMER 0.5. Primers were selected to have similar properties to facilitate standard conditions for PCR reactions. Primers are 20 to 23 nucleotides long with GC contents of 50-60% and melting temperature ranging from 59-64°C. Amplification and polymorphism for each EST was tested on 2% agarose gels. If length base polymorphisms were not revealed, then a set of 10 different restriction enzymes (*Hind*III, *EcoR*I, *Ava* II, *Bst*NI, *Dra*I, H*ae* III, H*in*f1, M*sp* I, *EcoR*V, R*sa* I) were tested to find restriction site based polymorphism among parents D89090-15 and F8909-17.

Objective 3

There are now a large number of EST derived SSR markers available, in addition to the genomic SSR markers from the Vitis Microsatellite Consortium. The EST derived SSR markers are more valuable if the cDNA sequence from which the EST was derived has a known function as determined by comparisons with homologs from other EST databases. We plan on selecting EST-SSR markers that show homology to genes which control disease resistance along with those that control other important morphological, physiological and agronomic traits. So far we have tested 90 EST-SSR markers from three different sources (Table 1) and 45 of informative markers were added to the entire core set of 9621 population. Our goal is to screen an additional 100-150 EST-SSR markers with putative known function and we are adding to the map as they are completed.

Objective 4

In order to develop the core framework map based on SSR markers, preliminary linkage analysis for each parent was carried out with MAPMAKER 2.0. Each segregating locus was paired with a "dummy" locus, resulting in a doubled data set. Linkage groups obtained from the doubled data set were then divided into two symmetrical sets of groups and one set was chosen for further detail. The "first order" and "compare" commands were used to determine the probable order of all markers in each linkage group. The integrated linkage analysis to obtain the sex-average map was performed with JOINMAP 2.0 (LOD 5.0 and recombination frequency 0.45). Using the fixed sequence command, the order of markers was determined relative to the established order obtained from the initial MAPMAKER analysis. Map units in centimorgans (cM) were derived from the Kosambi (K) mapping function. The integrated consensus map analysis was carried out with JOINMAP 3.0. The consensus linkage map was developed with 217 markers (155 SSR markers, 45 EST-SSR, 16 ESTP markers and the Pierce's disease resistance locus). A total of 214 markers fall in 19 linkage groups and only 3 markers were unlinked. Total map length is 1300 cM with average distance between markers of 5.9 cM. All markers were evenly distributed. The current map is depicted in Figure 1. The largest linkage group was comprised of 30 markers and smallest group consisted of 4 markers (Table 2). The locus for Pierce's disease resistance mapped to linkage group 14 with flanking markers on each side (Figure 1). Many additional markers have been added but have not been included on the map.

To saturate a narrow region around the *PdR1* locus resistance locus with molecular markers, the strategy of bulk segregant analysis (BSA) (Michelmore et al. 1991) in concert with the AFLP marker system has been initiated in cooperation with our report titled "Optimizing marker-assisted selection (MAS) for resistance to *Xylella fastidiosa* to accelerate breeding of PD resistant grapes." Work has begun within two segregating families from susceptible by resistant crosses. One family, C8909-07 by F8909-08, segregates 1:1 resistant to susceptible and a good correlation between resistance and resistance marker alleles has been established. A bulk of the DNA from the 12 most susceptible and a bulk of the DNA the 12 most

resistant genotypes are in process and will be tested for AFLP polymorphisms utilizing florescent primers and visualized on a PE 3100 sequencer.

Table 1. Data on number of markers mapped for the 9621 (D8909-15 x F8909-17) mapping population.

Molecular Markers		
Genomic SSR	VMC published/unpublished	134
	VVMD	10
	VVS	2
	INRA	9
EST derived SSR	Southern Cross University, Australia	4
	INRA, France	7
	Genome Facility (U.C. Davis)	35
ESTP markers	Doug Adams/NCBI data base	16
Grand Total		217

Table 2. Details of the 9621 genetic linkage map.

Linkage groups	19
Linked markers	214
Total map length	1300 cM
Average distance between markers	5.98 cM
Largest group (PD linkage group)	30 markers 80cM (group14)
Smallest group	4 markers 18cM (group 15)

Figure 1a. Riaz & Walker2004 SSR based genetic linkage map of 9621 (8909-15 X8909-17)

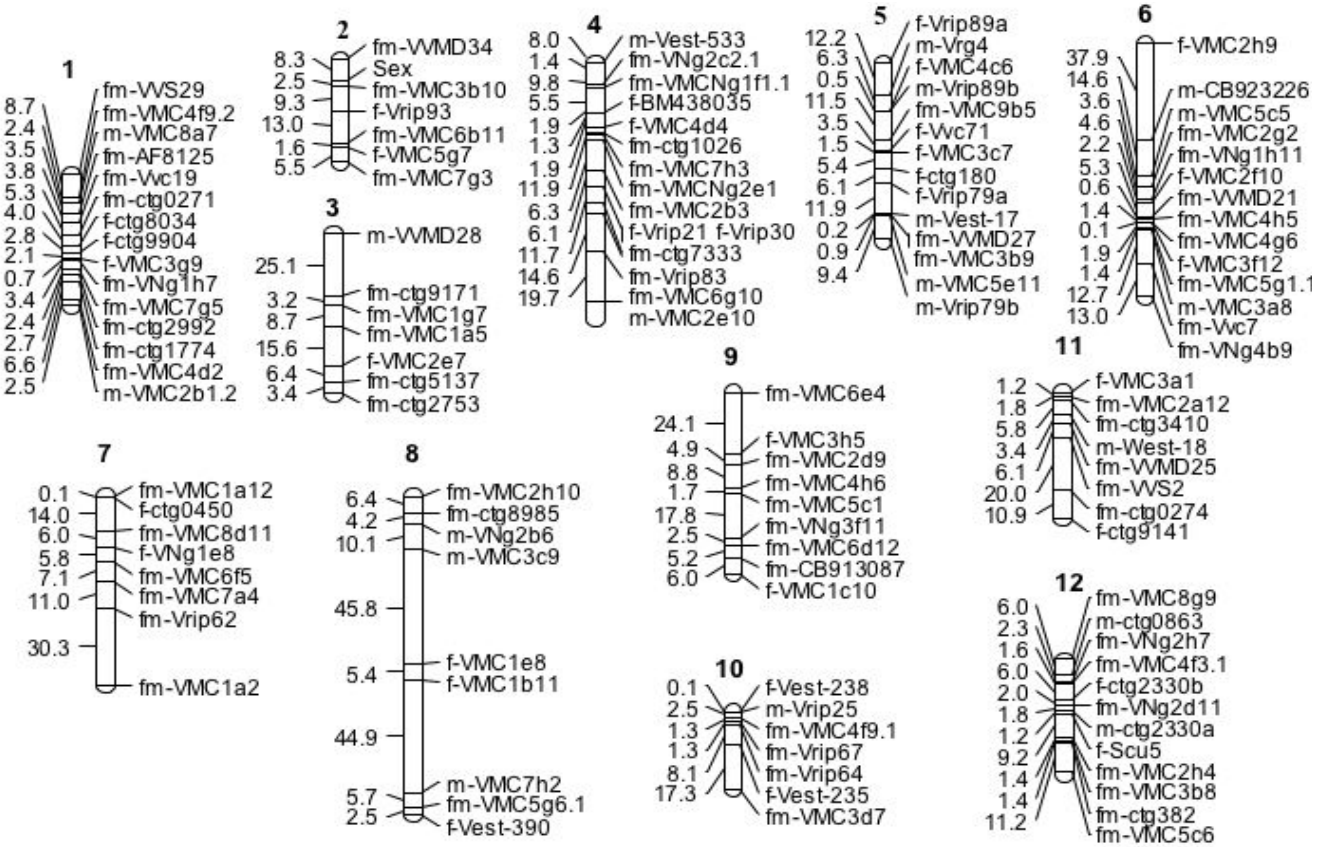
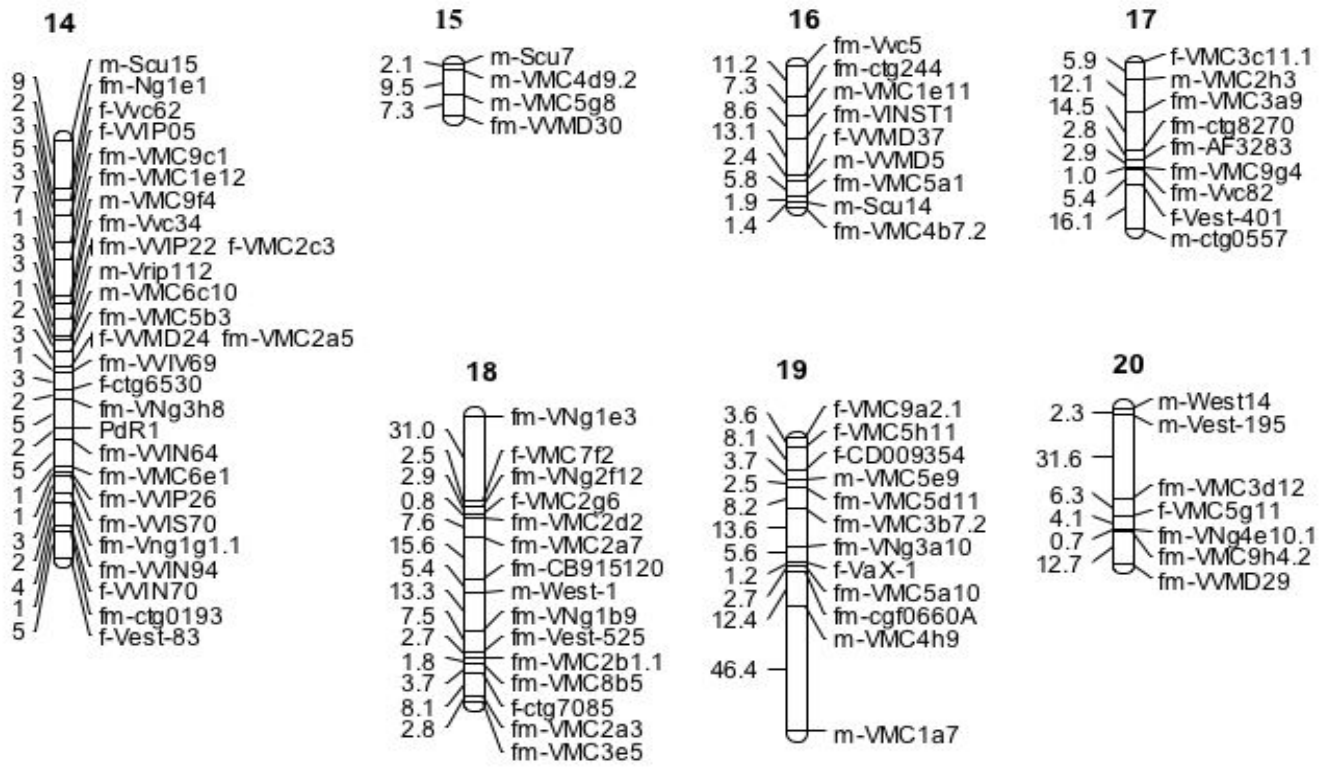


Figure 1b. Riaz & Walker2004 SSR based genetic linkage map of 9621 (8909-15 X8909-17)



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FUNDING AGENCIES

Funding for this project was provided by the CDFA Pierce's Disease and Glassy-winged Sharpshooter Board. Previous mapping efforts upon which this research is based received funding from the American Vineyard Foundation, the California Grape Rootstock Improvement Commission, and the Louis P. Martini Endowed Chair in Viticulture.

BREEDING PIERCE'S DISEASE RESISTANT WINEGRAPES

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Reporting Period: The results reported here are from work conducted from November 2003 through October 2004.

ABSTRACT
Strong and continued progress is being made in breeding Pierce's disease (PD) resistant grapes. Fruit quality has markedly improved while maintaining high levels of PD resistance. We continue to make many crosses, produce thousands of seeds, and plant about two thousand plants in the field each year. We have been increasing the number of seedlings and high fruit quality selections we test under our greenhouse screen. This screening is very severe, but material that passes the screen is reliably resistant and dramatically restricts *Xylella fastidiosa (Xf)* movement. We are also co-screening for powdery mildew resistance. The heritability of *Xf* resistance from a range of resistant southeast US (SEUS) cultivar and species parents is not consistent – some parents produce few resistant offspring, while others produce a large percentage – making careful parental screening very important. We have been able to expand our *Xf* screening the past few years and have tested hundreds of potential parents before we need to make breeding decisions the following year.

INTRODUCTION
Renewed and intensified PD outbreaks in historic PD zones in wine regions around the state and the introduction of GWSS into the southern San Joaquin Valley demonstrate the vulnerability of *V. vinifera* wine grape culture in California. All of California's wine grapes are susceptible to PD and no effective prevention or cure currently exists. Under severe PD pressure, culture of *V. vinifera* grapes is not possible. We are currently breeding PD-resistant wine grape cultivars for localized use in traditional PD "hot-spots" that are common in the North Coast, and it is likely that acceptable white and red wine grapes for these areas can be produced in two generations of crosses with our current *Xf* resistant selections. To further improve the utility of these *Xf* resistant cultivars, we are co-selecting for high levels of powdery mildew resistance. Unlike wine varieties for widespread use where the need for "pure *V. vinifera*" cultivars is enforced by marketing, given adequate quality (neutrality, color, season, cultural characteristics) varieties for localized use should prove useful to industry as blenders and by keeping "hot-spot" vineyard acreage in production. Our concurrent efforts to identify *Xf* resistance genes (see companion proposal – Walker and Riaz) will make it possible in the future to transform wine grapes with grape-derived resistance genes. Using grape genes to transform grapes should help overcome public reluctance about GM grapes and provide durable PD resistance.

PD resistance exists in a number of *Vitis* species and in the related genus, *Muscadinia*. Resistant cultivars have been developed in public and private breeding programs across the southeastern United States (SEUS). These cultivars have high PD resistance, but relatively low fruit quality relative to *V. vinifera* grapes. In the southeastern US, they must also resist downy and powdery mildew, black rot and anthracnose, which have as great an effect on viticulture in the southeast as PD does. Most of these diseases are not found in California, allowing breeders to incorporate more high quality *V. vini*fera into their breeding efforts and enabling the production of much higher quality PD resistant cultivars in a shorter time span. We have characterized (see past reports) and employed a wide range of PD resistant germplasm from the collections at the National Clonal Germplasm Repository, Davis; selections obtained from breeders in the southeastern U.S.; from *V. rupestris* x *V. arizonica* selections that have exceptional PD resistance; and from several *V. vinifera* x *M. rotundifolia* hybrid winegrape types that have some fertility. These breeding efforts have already resulted in relatively high quality selections with excellent PD resistance.

At UC Davis we are uniquely poised to undertake this important breeding effort. We have developed rapid screening techniques for *Xf* resistance and have optimized ELISA and PCR detection of *Xf* (Buzkan et al. 2003, Buzkan et al. 2004, Krivanek et al. 2004, Krivanek and Walker 2004). We have unique and highly resistant *V. rupestris* x *V. arizonica* selections, as well as an extensive collection of southeastern grape hybrids, that offer the introduction of extremely high levels of *Xf* resistance into commercial grapes. We also have several years' worth of seedlings in the ground that need evaluation as winegrape types.

OBJECTIVES
The objectives of our PD breeding project are divided into two primary parts. The first is the breeding of *Xf* resistant wine grapes through backcross techniques using *V. vinifera* wine grapes and *Xf* resistant selections and sources characterized from our previous breeding efforts. The second is the continuing characterization of *Xf* resistance and winegrape quality traits (color, tannin, ripening dates, flavor, productivity, etc.) in novel germplasm sources, in our breeding populations, and in our genetic mapping populations. These efforts support both the breeding program and the genetic mapping program.

Completion of these objectives is tied to the speed with which seedlings can be produced, fruited and evaluated and subsequent generations produced.

- Develop multiple lines of *Xf* resistant wine grapes using 8909 (*V. rupestris* x *V. arizonica* selections; *Xf* resistant breeder selections (DC1-39, Zehnder selections, etc); and southern grape species (*V. arizonica*, *V. champinii*, *V. shuttleworthii*, *V. simpsonii*, *M. rotundifolia*, and others).
- Continue backcross generations with 8909-08, DC1-39, and other lines to advanced *vinifera* selections and select for high quality wine grape characteristics.
- Continue to identify and characterize additional sources of *Xf* resistance with high levels of powdery mildew resistance.
- Maintain current and produce additional populations for genetic mapping efforts aimed at characterizing *Xf* resistance genes, and identifying and mapping fruit quality traits such as color, tannin content, flavor, production, etc. in *Xf* resistant backgrounds.
- Study the inheritance of *Xf* resistance from a broad range of resistance sources.

RESULTS AND CONCLUSIONS

Shift From Table Grape Breeding to Wine Types

Because the California Table Grape Commission's decision to not fund the breeding of PD resistant grapes, as of May 2004 we are now solely breeding PD resistant wine grapes. This year we evaluated 4,042 seedlings from 39 different crosses made in the last three years for use as wine grapes. From this number, four subgroups based on different resistance source were identified as particularly promising (Table 1). Promise was based on resistance to *Xf* and powdery mildew, fruit quality parameters, and viticultural characteristics such as yield and growth habit.

Evaluation of Fruit Quality

Within a cross we observed useful segregation of wine grape quality factors such as quality and quantity of color, acidity, pH, flavor, and skin and seed tannin. Table 2A and 2B present data for typical genotypes from three of the four resistance groups. These were harvested on August 26, 2004. Figure 1 displays clusters from two of the four promising *Xf* resistance subgroups listed in Table 1. Their morphology is becoming very vinifera-like in the first generation. Figure 2 displays juice extracted from some of the *Xf* resistant crosses in comparison with the juices from Cabernet Sauvignon and Pinot noir. There are a wide variety of colors that should allow matching enological needs with our selection process.

Planting of 2003 Crosses

Table 3 summarizes the field planting of wine crosses made in 2003. We did not germinate the 2,150 seeds of the cross of a SEUS cultivar by Syrah since our GH screening of progeny from the same SEUS female by pure *V. vinifera* indicated only 1 in 12 of the seedlings was likely to be resistant. Crosses made in Spring 2003 contained efforts directed at table and raisin grape production. This year's crosses were entirely devoted to wine grape efforts.

Wine Crosses Made in 2004

Table 4 details the wine grape crosses made during Spring 2004. We were able to tailor our choices for PD resistant parents with our previous experiences directed at table grape breeding. The assays of subsets of progeny from crosses with various parental sources found that the expression of PD resistance in progeny varies. *Vitis arizonica/candicans* selections from near Monterey, Mexico (b43-17, b43-36, and b43-56) produced 100% resistant progeny in the testing of the subset and should therefore be homozygous resistant. F8909-08 and F8909-17 were both derived from b43-17. The heritability of selections from Florida varied: BO2SG, BD5-117 and Midsouth produced 50% resistant progeny; while only 20% of the progeny of BO3SG was resistant, so progeny from it will be planted sparingly. NC-11J x UCD0124-01 represents a resistant x resistant cross from two different resistant backgrounds. B55-1 and NC6-15 are opportunities to ingress resistance from *Muscadinia rotundifolia* into wine crosses. We plan to plant between two and three thousand of the most promising seedlings from the crosses detailed above in Spring 2005.

Greenhouse Screen Results

We screened 474 genotypes with our greenhouse screen. The tested genotypes included cultivars and species from the SEUS, many Olmo Vinifera/Rotundifolia (VR) hybrids with potential PD resistance and for use as parents, table and wine grape crosses, and possible *Xf* resistant wine grape selections from a private breeder in North Carolina. Several promising *Xf*-resistant SEUS genotypes were identified. Six of 19 Olmo VR hybrids tested resistant. Two may be promising parents. None of the wine grape selections from North Carolina proved to be adequately resistant.

Table 5 presents the ratio of resistant to susceptible (R:S) progeny from crosses of highly susceptible *V. vinifera* parents crossed with a variety of *Xf* resistance sources. One *V. smalliana* and one *V. champinii* F1 hybrid progeny had R:S ratios of close to 1:1, suggesting that the resistance in these parents was heterozygous and controlled by a single gene. Other parents had ratios ranging from 1:3 through 1:11. Details are summarized in Table 5. We made crosses onto the *V. champinii* hybrid this year and they will be tested to see if the inheritance ratio remains 1:1, as does our F8909-17 resistance source (see Walker-Krivanek report). In other backgrounds, resistance seems to erode with continued backcrossing to *V. vinifera*, thus these stable resistance sources are very valuable and are easily adapted to marker-assisted selection.

Progeny from crosses of field resistant parents, like JS23-416 – judged resistant in Florida (Herb Barrett, personal communication) yet has been susceptible in our greenhouse tests, to *V. vinifera* do not seem to be resistant (<100,000 fu/ml). However, they do produce a broad and relatively even distribution of progeny from 170,000 to almost 6,500,000 cfu/ml. Although we would not consider those at the low end of this scale to be resistant, they have as low or lower bacterial levels than do some of the field resistant genotypes from the SEUS we have tested. We have avoided these progeny and using these parents to prevent release of field resistant cultivars that may survive PD infection, but allow vine-to-vine movement in vineyards.

We are beginning testing of about 200 genotypes with results expected in March 2005. These results will be used to direct backcrossing of the most resistant genotypes to *V. vinifera* wine grapes.

Napa Field Trial

This year we planted another block in our field trial at Beringer Vineyards in Yountville. We expanded the plot by adding 6 vine replicates of 20 different genotypes from 4 different resistant sources. Based on our GH screen results, both highly resistant and highly susceptible genotypes from each resistant source were planted. These will be inoculated with *Xf* next April and ELISA tested in October 2005.

This fall we observed the most pronounced visual PD symptoms to date in the 2001 and 2003 plantings following inoculation with *Xf* early this spring. We used a mixture of 5 different Napa PD strains as inoculum. The 2001 planting consists of known field resistant selections from the SEUS, and the 2003 planting consists of 3 vine reps of some of our early crosses and a few more SEUS field resistant types. On October 8, 2004 we scored these vines for visual symptoms and took samples for ELISA testing from 291 vines in these blocks. Results will be reported in December.

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FUNDING AGENCIES

Funding for this project was provided by the CDFA Pierce's Disease and Glassy-winged Sharpshooter Board. In the past, funding has also been received from the California Raisin Marketing Board, the California Table Grape Commission, and the USDA Animal and Plant Health Inspection Service.

Table 1. Summary of different crosses within the subgroups and the relative number of genotypes within each group that merit further evaluation.

Resistance Source	*V. vinifera* Parent	Genotypes Evaluated	Genotypes Selected
BO2SG (*V. smalliana*)	C1020	36	10
	Princess	21	9
BO3SG (*V. smalliana-simpsonii*)	C67-129	30	7
	Princess	81	14
AW C52-94 (*V. simpsonii*)	C51-63	353	71
Midsouth	B90-116	39	4
	C67-129	46	1
	Princess	8	1
Total		614	117

Table 2A. Analytical evaluation of representative progeny from three different sources of *Xf* resistance.

Genotype	Species or Cross	Cluster Wt. (g)	Brix	pH	TA (g/L)	Berry Wt. (g)	Est. Yield (gal/ton)
BO2SG	*V. smalliana*	45	24.5	3.28	19.7	0.3	129
BO3SG	*V. smalliana-simpsonii*	66	25.0	3.53	12.1	0.3	90
Cab Sauv	*V. vinifera*	269	23.0	3.52	6.8	1.0	160
Pinot noir	*V. vinifera*	299	25.5	3.72	6.1	1.2	182
J13-09	BO2SG x Melissa	184	24.2	3.16	12.1	1.3	160
J13-13	BO2SG x Melissa	62	25.5	3.22	9.8	1.4	162
J14-09	BO2SG x C1020	90	25.2	3.36	9.1	1.2	176
J14-12	BO2SG x C1020	125	27.0	3.46	8.3	1.0	167
J14-16	BO2SG x C1020	120	26.0	3.38	9.8	1.4	170
J17-3	BO3SG x C67-129	100	25.0	3.32	7.1	1.3	150
J17-06	BO3SG x C67-129	102	25.8	3.53	6.4	1.4	149
J17-08	BO3SG x C67-129	117	26.5	3.43	7.7	1.0	135
J17-14	BO3SG x C67-129	200	27.0	3.68	5.9	0.9	148
J17-24	BO3SG x C67-129	224	26.0	3.62	6.7	1.1	137
J17-25	BO3SG x C67-129	70	27.0	3.65	5.9	1.0	146
J17-36	BO3SG x Melissa	110	26.5	3.76	4.5	0.9	154
J17-39	BO3SG x Melissa	70	25.0	3.33	7.4	0.8	176
J17-50	BO3SG x Melissa	185	24.0	3.32	6.8	1.2	165
J18-18	BO3SG x Melissa	195	23.0	3.14	9.8	1.1	143
J18-24	BO3SG x Melissa	60	26.5	3.54	5.5	1.1	148
J18-35	BO3SG x Melissa	93	26.2	3.55	6.2	0.9	152
J18-37	BO3SG x Melissa	100	23.5	3.14	9.7	0.7	158
J18-38	BO3SG x Melissa	101	25.0	3.23	8.6	1.0	154
J27-03	Midsouth x B90-116	99	23.5	3.85	8.3	1.2	168
J27-06	Midsouth x B90-116	125	25.0	3.76	5.2	1.2	145

Table 2B. Sensory evaluation of representative progeny from three different sources of *Xf* resistance.

Genotype	Species or Cross	Skin Tannin Intensity[a]	Seed Color[b]	Juice Hue	Juice Color Intensity	Juice Flavor
BO2SG	*V. smalliana*	2	4	red	dark	fruity, peppery
BO3SG	*V. smalliana-simpsonii*	1	4	red	dark	fruity, peppery
Cab Sauv	*V. vinifera*	3	2.5	pink	light	slightly vegetal
Pinot noir	*V. vinifera*	1	4	pink	very light	fruity
J13-09	BO2SG x Melissa	2	4	red	medium +	tart, red fruit
J13-13	BO2SG x Melissa	2.5	4	red-purple	medium +	fruity, slight hot pepper
J14-09	BO2SG x C1020	2	4	red	medium	tart, jammy, very slight hot pepper
J14-12	BO2SG x C1020	2	4	pink	light	slightly jammy, broad fruity
J14-16	BO2SG x C1020	2	4	green		green pepper, hot pepper
J17-3	BO3SG x C67-129	1.5	4	red-purple	medium +	slightly fruity, hot pepper
J17-06	BO3SG x C67-129	2	3.5	pink-red	medium	hay, hot pepper
J17-08	BO3SG x C67-129	1.5	4	pink-orange	light +	vinifera-like, acidic, hot pepper
J17-14	BO3SG x C67-129	2	4	red	medium	slightly jammy, fruity
J17-24	BO3SG x C67-129	4	4	red	medium +	fruity, hot pepper
J17-25	BO3SG x C67-129	1.5	4	red	medium	very slightly vegetal-herbal
J17-36	BO3SG x Melissa	2	4	pink	medium -	slight hay, hot pepper
J17-39	BO3SG x Melissa	2	4	red	medium +	tart, raspberry, very slight hot pepper
J17-50	BO3SG x Melissa	2	4	pink-red	medium	simple fruit, berry
J18-18	BO3SG x Melissa	3	4	pink-red	medium -	slight hay, canned
J18-24	BO3SG x Melissa	2	4	red	medium	slight hay, fruity
J18-35	BO3SG x Melissa	2	3.5	pink-red	medium -	hay, hot pepper
J18-37	BO3SG x Melissa	2	4	pink-brown	light	tart berry, slightly buttery
J18-38	BO3SG x Melissa	1	4	red	medium -	berry, slight hot pepper
J27-03	Midsouth x B90-116	1	4	purple	dark	current, vegetal
J27-06	Midsouth x B90-116	1	4	red	medium-	strawberry, herbal

a = (1=low, 4= high); b = (1=green, 4= brown)

Table 3. UC Davis field plantings of wine crosses made in 2003. F2-7 and F2-35 are respectively a black and a white female seedling of the cross Cabernet Sauvignon x Carignane. B34-82 is a USDA cross.

Cross	Resistance Source	Seedlings Planted
F2-7 x F8909-08	*V. arizonica*	10
F2-35 x F8909-08	*V. arizonica*	38
F2-35 x BD5-117	SEUS complex	164
F2-7 x BD5-117	SEUS complex	149
BD5-117 x B34-82	SEUS complex	141
	Total	502

Table 4. Wine grape crosses made at UCD in 2004.

Female Parent	Male Parent	Resistance Source	# Seeds
BO2SG	Cabernet Sauvignon	*V. smalliana*	376
BO2SG	Carignane	*V. smalliana*	196
BO2SG	Sauvignon blanc	*V. smalliana*	404
BO3SG	Chambourcin	*V. smalliana-simpsonii*	412
BO3SG	Petite Sirah	*V. smalliana-simpsonii*	419
BO3SG	Cabernet Sauvignon	*V. smalliana-simpsonii*	371
BO3SG	Carignane	*V. smalliana-simpsonii*	350
BO3SG	Sauvignon blanc	*V. smalliana-simpsonii*	223
F2-7 (CabS x Carig.)	BD5-117	SEUS complex	1131
F2-7	Midsouth	*V. champinii*	522
F2-7	F8909-08	*V. arizonica - candicans*	4,500
F2-7	F8909-17	*V. arizonica - candicans*	300
F2-35 (CabS x Carig.)	B55-1	*M. rotundifolia*	18
F2-35	B43-17	*V. arizonica-candicans*	323
F2-35	B43-36	*V. arizonica*	141
F2-35	B43-56	*V. arizonica*	56
F2-35	BD5-117	SEUS complex	783
F2-35	Midsouth	*V. champinii*	522
NC-11J	UCD0124-01	*M. rotundifolia*-SEUS complex	175
Midsouth	Midsouth	*V. champinii*	500
NC6-15	Sauvignon blanc	*M. rotundifolia*	50
Total			11,772

Table 5. Ratios of *Xf*-resistant: susceptible (R:S) progeny in populations from various resistance sources by *V. vinifera* parents based on a greenhouse screen. Resistance is defined as a mean value less than 100,000 cfu/ml (colony forming ***units per ml).***

Resistant Parent	Resistance Source	Number Resistant	Number Tested	Percent Resistant	Approx: R/S ratio
Midsouth	*V. champinii*	9	17	53%	1:1
BO2SG	*V. smalliana*	11	23	48%	1:1
Cha3-48	*V. champinii*	8	26	31%	1:2
DC1-39	Complex	9	33	27%	1:3
BO3SG	*V. smalliana-simpsonii*	1	6	17%	1:5
F901	*V. shuttleworthii*	1	7	14%	1:6
AW c52-94	*V. simpsoni*	2	15	13%	1:6
Z 71-50-1	Complex	2	25	8%	1/11
AT0023-019	*V. arizonica* (La Paz)	2	29	7%	1/11
F902	*V. shuttleworthii*	0	16	0%	-
Roucaneuf	Complex	0	22	0%	-
Villard blanc	Complex	0	6	0%	-
JS23-416	Susceptible	0	19	0%	-
Total			244		

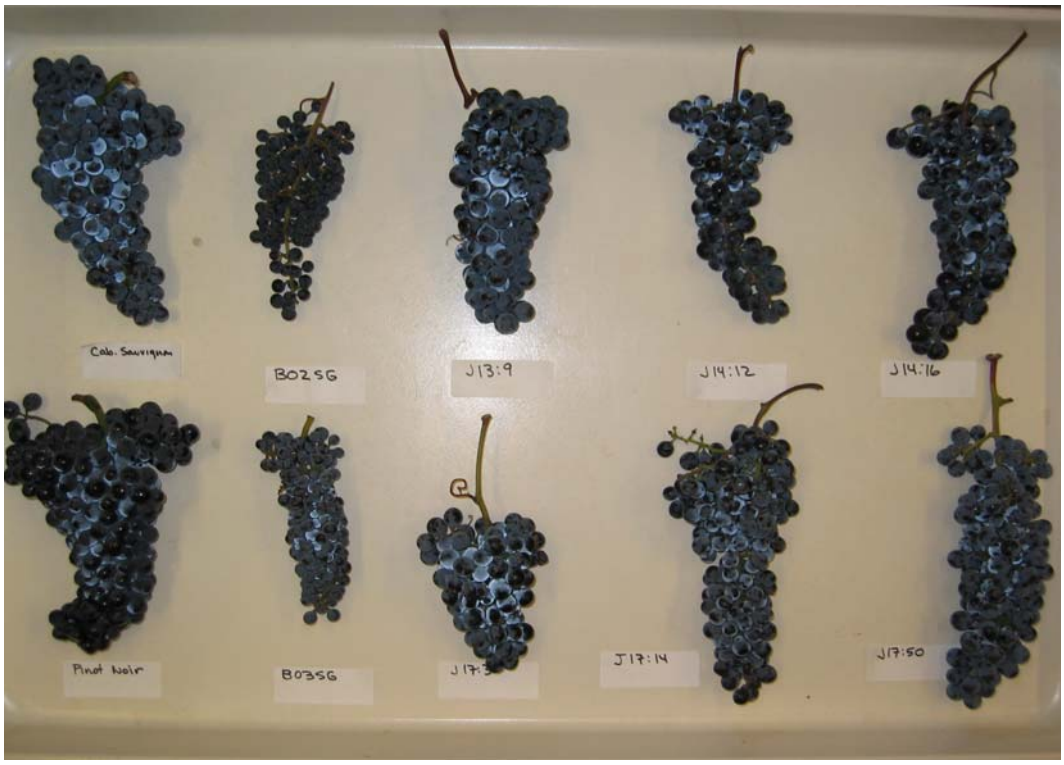

Figure 1. Representative clusters from two promising *Xf* resistance source subgroups. BO2SG and BO3SG are the resistant female parents. Cabernet Sauvignon and Pinot noir are shown for size/shape comparisons. Crosses to BO2SG are in the top row while crosses to BO3SG are in the bottom row. The other clusters are from first generation crosses. Analytical details can be found in Table 2.

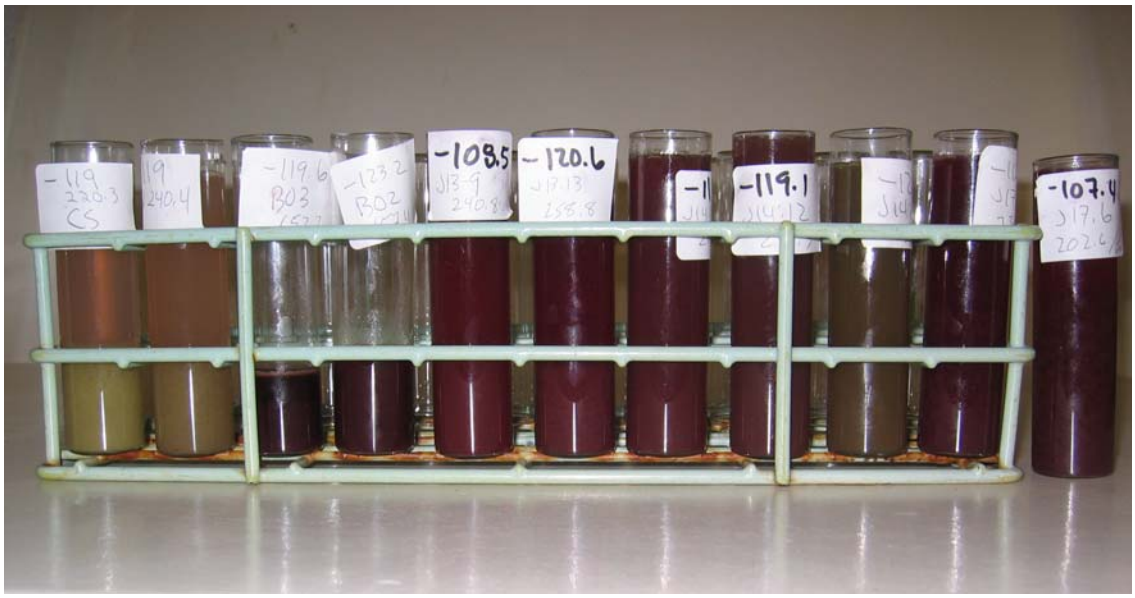

Figure 2. Juice extracted from selected clusters of *Xf*-resistant crosses shown in Figure 1 and detailed in Table 2. Note the high quantity of red color and the variation in hue from some of the crosses. This variation allows for tailoring varieties to meet particular enological needs. Juice from Cabernet Sauvignon and Pinot noir are on the left in the first two vials respectively.